Agilent CC-Mode Software

User's Guide

Agilent Technologies

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User's Guide

Table of Contents

Chapter 1 **Installing CC-Mode Software**

Describes how to install and start the CC-Mode software, 11

Before you start, 12
To install CC-Mode software, 13
Startup sequence - Sampler Mode, 15
Startup sequence - Sampler and Fraction Collection Mode, 16
    To change to Sampler/Fractionator Mode, 16
To start the CC-Mode software, 17

Chapter 2 **System Configuration**

Describes how to configure the CC-Mode software and the Agilent 220 Micro Plate Sampler, 19

System Configuration 20
To change the system configuration, 21
To change the rack configuration, 24
To design plates, 27
To design racks, 29
To change plate orientation and fill direction for fraction collection, 30

Chapter 3 **Sampler Initialization and Maintenance**

Describes how to initialize and maintain the Agilent 220 micro plate sampler, 31

Sampler Initialization, 32
To flush the syringe and tubing, 34
To test valve switching on the 220 micro plate sampler, 35
    High Pressure valve, 35
    Low Pressure valve, 35
To replace the syringe, 36

Chapter 4 **Study Setup**

Describes what a study is and how to set one up, including fraction collection and FIA analysis, 37

What is a study 38

To set up a new study for INB22000, 41
To create a new study, 42
- Method selection per injections, 43
- To add a method, 44
- Study layout, 44
- Study, 45
- Fractions, 45
- Sample name format, 45
- Data file path, 46
- Repo rting, 46
- Save as CSV files, 47
- Graphic output, 48
- Extended plate report, 48

To select samples for the study, 50
- Adding samples to a study manually, 50
- Importing sample data from another application, 51
- To remove samples from the study, 53

To add fractions, 54
To open or edit an existing study, 55
To select methods, 57
- To add a method, 57
- To replace a method, 57
- To delete a method, 57

To edit method parameters, 58
To view study lists, 59
To create a partial study, 60
- Interactively (from the Study Layout screen), 60
- Through a study list, 60

To set up for fraction collection, 61
To set up an FIA study, 64
To report study data and parameters, 66

Chapter 5 Sampler Tasks Setup

Describes how to set parameters on the Agilent 220 Micro Plate Sampler, 67

To set sampler parameters, 68
Sampler tasks reference, 70

Create Air Gap, 70
Draw Sample, 71
Inject Sample, 71
Centered Loop Fill (called Partial Loop Fill in the previous release), 72
Partial Loop Fill and Inject, 74
Rinse Needle Inside / Needle Outside / Injection Port, 75
Wash Vial, 75
Sample Preparation, 76
Draw from Destination, 78
Enable Fraction Collection, 78
Enable Fraction Collection Delay Sensor, 83
Examples of sampler task lists, 84
Standard Injection program, 84
Centered Loop Fill, 85
Sample Injection followed by Fraction Collection, 85
Sample Preparation (without sample injection), 86
Sample Preparation (with sample injection), 86
To optimize sampler performance, 88
Rinse Needle Inside/Outside, Rinse Injection port, Wash vial, 88
Draw and eject speed, 89
Injection Techniques – Loop Filling Modes, 89

Chapter 6 Study Setup - LC/MSD Data Analysis

Describes how to set up a study for LC/MSD Data Analysis, including mass-based fraction collection, 91

Study Setup for LC/MSD Data Analysis 92

To set up a ChemStation method to use with CC-Mode, 94
To set up study parameters - LC/MSD, 95
To set up purity calculations, 97
Purity calculation reference, 99
To specify the report format - LC/MSD, 100
To add expected masses to the study list, 102
To calculate the mass from the molecular formula, 104
To import expected masses from a CSV file, 106
To set up for fraction collection - LC/MSD, 109

Chapter 7 Data Acquisition and Fraction Collection

Describes how to run and monitor studies, fractions and FIA analyses, 111

To set up a ChemStation method to use with CC-Mode, 112
Reprocessing, 113
To set up a ChemStation FIA method to use with CC-Mode, 114
To run a study, 115
To monitor the status of a run, 117
Status screen, 117
Status bar, 117
To analyze fractions, 119
To automatically analyze fractions, 120
To pause or stop a study, 121
To pause a study, 121
To resume a study, 121
To stop a study, 121

Chapter 8 Data Review

Describes how to review analytical results and print or export them, 123

To review the results of a study, 124
To view the results of a study for individual samples, 125
To view the sample/fraction relation, 129
To review FIA results, 130
To reprocess a study, 132
To print or export results, 133
To review CC-Mode results remotely, 135

Chapter 9 Checkout Procedures

Describes how to perform the checkout procedure for the CC-Mode software and 220 micro plate sampler system, including fraction collection, 137

Checkout Procedures 138

System Checkout 140

Step 1 - Prepare the LC system, 142
Step 2 - Prepare the Agilent 220 micro plate sampler, 145
Step 3 - Create a new study, 146
Step 4 - Set study parameters, 147
Purity calculation settings (LC/MSD), 148
Study layout, 148
Study list, 149

Step 5 - Run the analysis, 150
Before you start, 150
Start the analysis, 150
Check the system status, 150
Observe the chromatogram, 151
Step 6 - Review LC/MSD results, 152

UV-triggered Fraction Collection Checkout 153

Step 1 - Prepare the LC system, 155
Step 2 - Prepare the Agilent 220 micro plate sampler, 157
Step 3 - Create a new study, 158
Step 4 - Set study parameters, 159
Study layout, 161
Step 5 - Run the analysis, 162
Before you start, 162
Start the analysis, 162

Mass-based Fraction Collection Checkout 163

Step 1 - Prepare the LC system, 165
Step 2 - Determine the collection delay time, 166
Step 3 - Prepare the 220 micro plate sampler, 168
Step 4 - Create a new study, 169
Step 5 - Set study parameters, 170
Study layout, 171
Step 6 - Run the analysis, 173
Before you start, 173
Start the analysis, 173
Step 7 - Set up mass-based fraction collection method, 174
Step 8 - Prepare the 220 micro plate sampler, 177
Step 9 - Create a new study, 178
Step 10 - Set study parameters, 179
Study layout, 182
Step 11 - Run the analysis, 183
Before you start, 183
Start the analysis, 183

1

Before you start, 12
To install CC-Mode software, 13
Startup sequence - Sampler Mode, 15
Startup sequence - Sampler and Fraction Collection Mode, 16
To start the CC-Mode software, 17

Installing CC-Mode Software

Describes how to install and start the CC-Mode software

Before you start

The tasks below must be completed prior to starting the software installation:

- ☐ **Install the following hardware.**
  - ☐ Agilent 1100 Series Liquid Chromatograph
  - ☐ Agilent 1100 Series LC/MSD (optional)
  - ☐ Agilent 220 Micro Plate Sampler
  - ☐ Fraction collection accessory (optional)
  - ☐ 35900E Analog to Digital Converter (optional)
- ☐ **Make all necessary electrical connections.**
- ☐ **Install the Agilent ChemStation computer and software revision A.07.XX or higher. Verify that the software is operational.**
- ☐ **Make sure that you are familiar with the 1100 Series LC, LC/MSD (if installed) and the ChemStation software.**

CAUTION Multi-instrument ChemStation configuration is not allowed when CC-Mode is running.

To install CC-Mode software

The CC-Mode software consists of the following products, some of which are optional and require separate software licenses:

- INB22000: Base software for control of the 220 micro plate sampler. Also controls the optional fraction collection accessory if G2251A hardware is installed.
- INB22002: LC/MSD data analysis software
- INB22003: to provide mass-based fraction collection capabilities
- INB22004: standalone LC/MSD data analysis software for use with an offline ChemStation. Can also be used with an online ChemStation.

Use the following procedure to install CC-Mode software and options.

1 **Verify that you have the CC-Mode product CD-ROM and the necessary license numbers, depending on the options ordered.**

2 **Start Windows NT 4.0 and close all open applications.**

3 **Insert the CC-Mode CD-ROM into the drive.**

4 **Using Windows NT Explorer, double-click on `setup.exe` on the CD-ROM to start the CC-Mode installation program.**

5 **When the Welcome screen appears, click Next to continue.**

6 **When the Select Components screen appears, select either INB22000 CC-Mode Base Software or INB22004 Standalone LC/MSD data analysis software and click Next.**

If you select INB22000, you will be prompted for additional installation options described in Step 7 below. Otherwise skip to Step 8.

7 **Select one or more of the following optional software components to install, if you have purchased these products. Select *all* options if you plan to do mass-based fraction collection. Click Next to continue.**

- INB22002: LC/MSD data analysis software
- INB22003: mass-based fraction collection

8 **You are prompted to enter the license number for each software component you have selected to install. Click Next to continue.**

See CC-Mode license documentation.

9 **When the Choose Destination Location dialog box appears, click Next to accept the default directory `C:\CCMode`, or click Browse to select a different location.**

NOTE The name of the installation directory for the CC-Mode software must not be longer than 8 characters and must be a valid file name.

During installation, files are copied from the CD-ROM to the destination directory and your Windows system is configured for use with the new software.

If you get warning messages during the installation that certain files and reports already exist, then the CC-Mode software may already exist in the specified directory. In this case, click Cancel to stop the installation. Uninstall the previous version of the software using the Windows NT Control Panel, then repeat the CC-Mode software installation.

10 **You are prompted to remove the CC-Mode software CD from the drive. You may choose to restart your computer now or later. Click Finish to complete the software installation.**

11 **If you plan to do fraction collection (optional G2251A hardware), install the Control Module as described in the Agilent 220 Micro Plate Sampler Reference Manual.**

12 **If you plan to do mass-based fraction collection (optional INB22003 software), install the required splitter and associated hardware as described in the "Agilent 220 Micro Plate Sampler Reference Manual", in addition to the Control Module described above.**

NOTE If you ever want to uninstall CC-Mode software, use the **Add/Remove Programs** utiltiy in the Windows Control Panel.

Startup sequence - Sampler Mode

After you install the CC-Mode software and the 220 Sampler hardware as described in the 220 Sampler Reference manual, follow the steps below to start your system in Sampler mode. Sampler mode is designed for high-throughput analysis. Samples can be injected from different types of sample containers such as well plates, vials and test tubes.

1 **Start up the 1100 LC and LC/MSD respectively.**

2 **Turn on the 220 Sampler and the injection valve.**

3 **Start the ChemStation software.**

CAUTION The ChemStation must be turned on *after* the 220 sampler.

4 **Access the Method & Run Control view in the ChemStation software.**

5 **If the CC-Mode menu is not currently displayed in the ChemStation Method & Run Control window, then select Full Menu from the View menu.**

6 **Select Start Software from the CC-Mode menu to start the CC-Mode software.**

CAUTION Only start the CC-Mode software from the ChemStation menu; *not* from the Windows Start menu.

Startup sequence - Sampler and Fraction Collection Mode

After you have installed the software as described in the previous section and the 220 Sampler hardware and fraction collection accessory and Control Module as described in the 220 Sampler Reference manual, follow the steps below to start your system in Sampler and Fraction Collection mode. Sampler and Fraction Collection mode enables the sampler to take samples from various sample containers and collect fractions into other sample containers.

1 **Start up the 1100 LC and LC/MSD respectively.**

2 **Turn on the 220 Sampler, sample injection valve and Control Module.**

3 **Start theChemStation software.**

4 **Access the Method & Run Control view in the ChemStation software.**

5 **If the CC-Mode menu is not currently displayed in the ChemStation Method & Run Control window, then select Full Menu from the View menu.**

6 **Select Start Software from the CC-Mode menu to start the CC-Mode software.**

By default the software is configured to Sampler Only Mode. Follow the directions below to change to Sampler/Fractionator Mode.

To change to Sampler/Fractionator Mode

1 **Select System Configuration from the Configuration menu in the CC-Mode Main window to display the System Configuration dialog box.**

2 **Select the Sampler/Fractionator Mode option; click OK.**

3 **Exit the CC-Mode window and start the software again from the ChemStation menu bar.**

Once the CC-Mode software has started to load, a message appears briefly to indicate that code is being downloaded to the sampler Control Module.

See also

To change the system configuration, 21

To start the CC-Mode software

The standard ChemStation for LC and LC/MSD systems has been extended with the addition of CC-Mode software, dedicated to control the 220 Sampler.

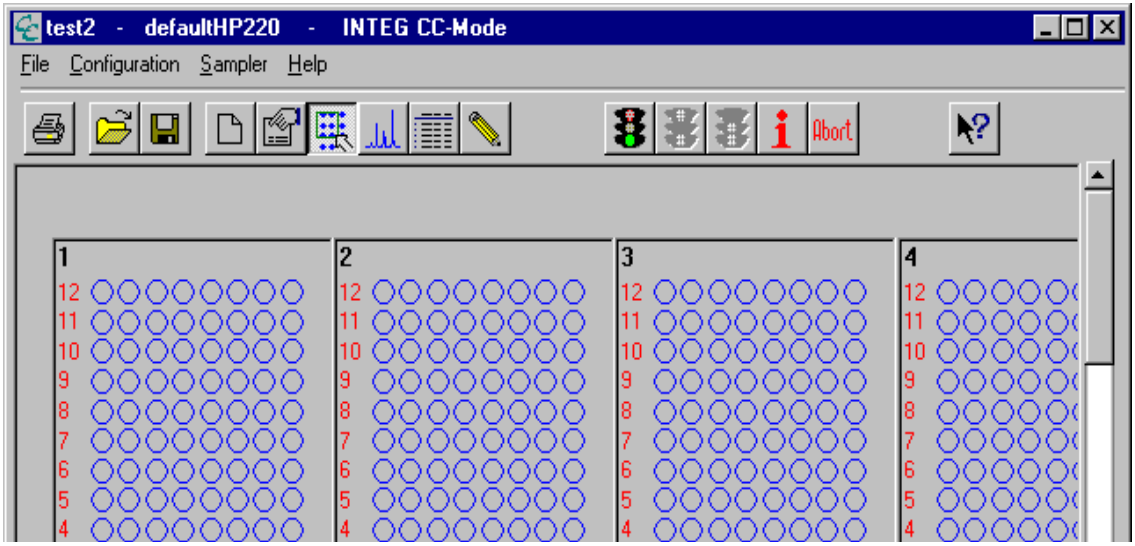
1 **Access the Method & Run Control view of the ChemStation software.**

2 **If the CC-Mode menu is not currently displayed in the ChemStation Method & Run Control window, then select Full Menu from the View menu.**

3 **Select Start Software from the CC-Mode menu.**

The CC-Mode Main window appears showing the Study Layout screen. Use the Study Layout screen to perform the following tasks:

- select samples and fractions for analysis
- display an overview of results
- view the plates used for samples, fractions and sample preparation



See also

See the CC-Mode online help for a description of the toolbar buttons and menu items in the CC-Mode software.

To start the CC-Mode software

2

To change the system configuration, 21
To change the rack configuration, 24
To design plates, 27
To design racks, 29
To change plate orientation and fill direction for fraction collection, 30

System Configuration

Describes how to configure the CC-Mode software and the Agilent 220 Micro Plate Sampler

System Configuration

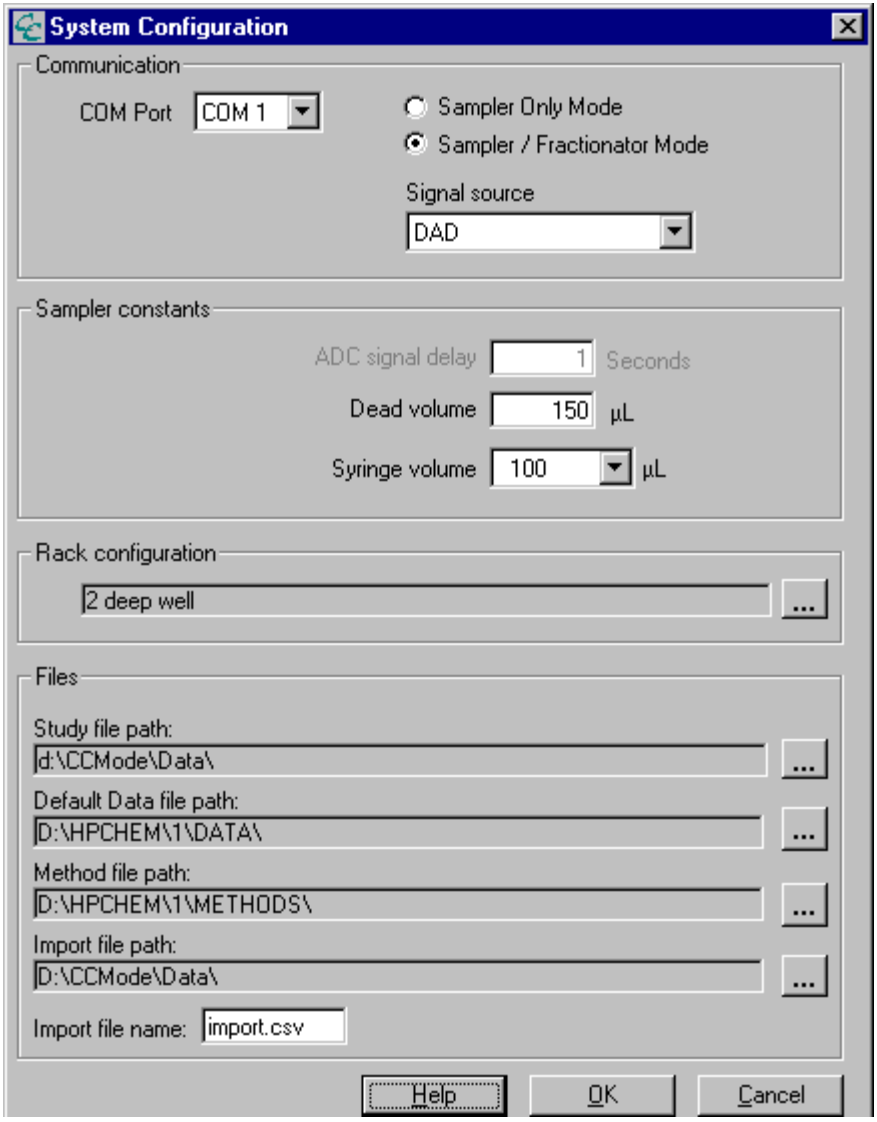
Once the CC-Mode software is running, the software must be configured as described in this chapter. System configuration is required the first time the software is used. Otherwise, it can be used later to make changes, such as selecting different types of racks, syringes or file paths.

Operating modes for CC-Mode software

- Sampler Only mode lis specifically designed for high-throughput analysis. In this mode, samples can be injected from various sample containers such as well plates, vials and test tubes using the Agilent 220 Micro Plate Sampler. This is the mode that the software is in unless you change it.
- Sampler/Fractionator mode (requires optional fraction collection accessory hardware) enables the 220 Micro Plate Sampler to take samples from various sample containers and collect fractions into other sample containers. With the optional INB22002 and INB22003 software, the fraction collection can be triggered by the detection of user-selected masses.

To change the system configuration

1 **Select System Configuration from the Configuration menu in the CC-Mode Main window to display the dialog box shown below.**



2 Use the System Configuration dialog box to change any of the following configuration parameters:

Communication

COM 1 is the default COM port.

Sampler Only Mode is the default mode. Select Sampler/Fractionator Mode if you have the optional hardware and software for either UV-based or mass-based fraction collection.

For Sampler/Fractionator Mode, select the appropriate signal source to use for peak detection. Note that only 1100 series DAD, VWD, FLD detectors are supported, plus the 35900E Analog to Digital Converter used to connect other detector analog signals such as the Agilent MWD.

Sampler Constants

ADC signal delay is the time delay caused by the detector connected to the AD Converter. The time delay is the time measured between moment when a peak enters the detector and moment of the corresponding signal change at the analog output of the detector. This field is enabled only if you select both the Sampler / Fractionator Mode and Signal source ADC.

The dead volume parameter is used for fraction collection and is the volume of the tubing that connects the detector cell outlet with the low pressure (switching) valve of the Agilent 220 Micro Plate Sampler (default volume = 150 µl). See the "Agilent 220 Micro Plate Sampler Reference manual" for more information.

The syringe volume parameter lets you select the syringe size for the dilutor. The default size is 100 µl. You must change the syringe to inject larger volumes. If you use a larger syringe, you must also use a sample loop greater than the default 5 µl loop on the Rheodyne valve.

.Rack Configuration

Allows you to select the rack composition. The racks hold the vials, test tubes, and plates. By default the Agilent 220 Micro Plate Sampler is equipped with one 505H rack for up to 10 well plates and one 205H rack for up to two well plates. Other rack configurations you created in the Rack Configuration screen also appear in this list.

CAUTION

The rack configuration selected here must match the actual configuration on the sampler exactly. The rack configuration is stored with each study when the study is created. When a study is loaded with a rack configuration that does not match the current rack configuration, the system does not allow you to perform actions involving the sampler.

CAUTION

Test a new rack configuration with a loosened injector needle to avoid damage prior to starting a study.

File Paths

Allows you to specify the global paths for studies, data files, method files and for import files. These directory paths will be used for all *future* studies. The paths for previously acquired studies will *not* be retroactively changed. In reprocessing a study acquired under a different setting, you will need to manually specifiy the paths where the files are actually located or move the files to the new defaults. Ensure that the correct path is selected to communicate with the ChemStation.

CAUTION

Method and data file paths cannot contain directory names longer than eight characters.

Import file name

Used to set the name of the file to import study sample list from. The default import file name is `import.csv`.

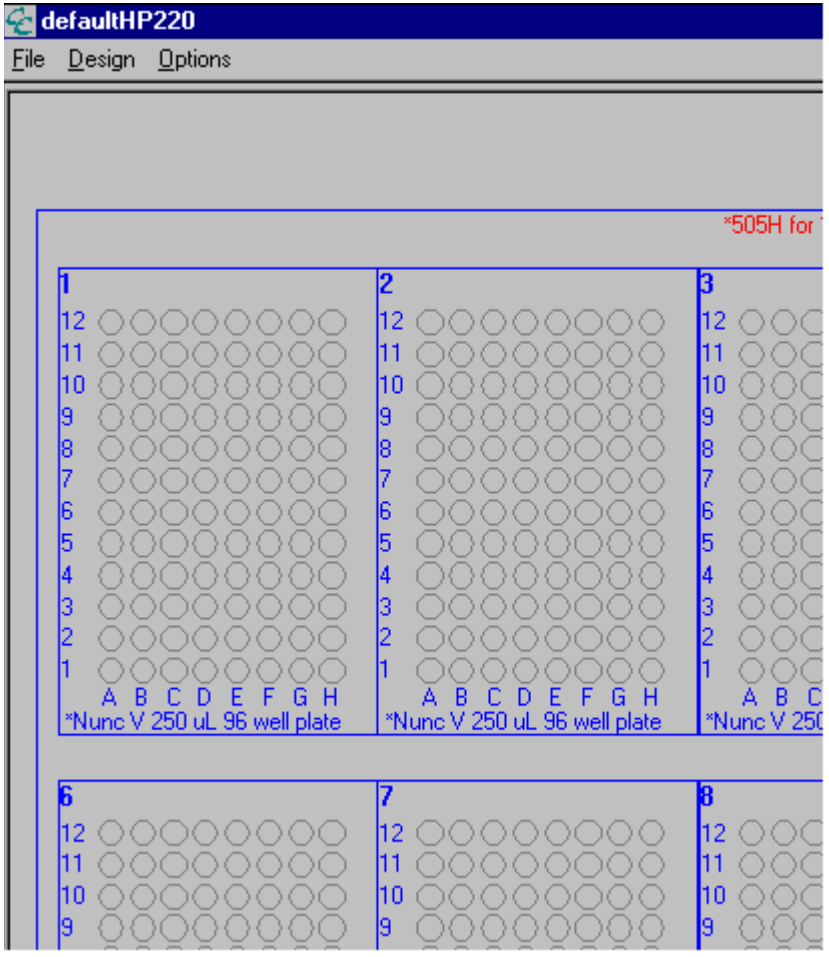
3 After you have made the desired configuration changes, click `OK` to close the System Configuration dialog box.

4 Exit CC-Mode, then restart it from the ChemStation software to activate the new system configuration settings.

To change the rack configuration

1 Select Rack Configuration from the Configuration menu in the CC-Mode Main window.

The Rack Configuration window appears (partial screen shown below).



2 Select one of the following options from the File menu:

New Configuration To create a new rack configuration. The default 220 Sampler window shows an

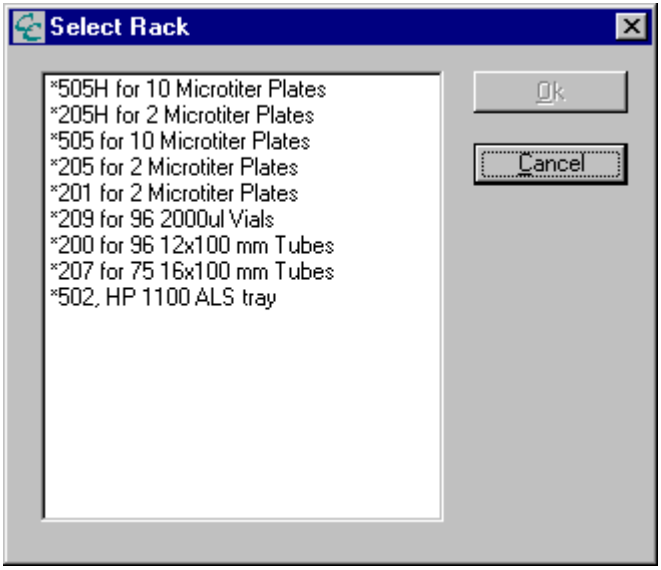
outline representing the sampler's work area. Five rectangles identify the empty rack locations.

Open Configuration

To open an existing configuration for review or editing.

3 To specify a new rack, click with the right mouse button on the desired rack location. Select Place Rack from the pop-up menu.

The Select Rack dialog box shown below appears. The racks in the list can be used as templates to customize the racks or to create similar racks.


Select Rack
*505H for 10 Microtiter Plates
*205H for 2 Microtiter Plates
*505 for 10 Microtiter Plates
*205 for 2 Microtiter Plates
*201 for 2 Microtiter Plates
*209 for 96 2000ul Vials
*200 for 96 12x100 mm Tubes
*207 for 75 16x100 mm Tubes
*502, HP 1100 ALS tray
Ok
Cancel

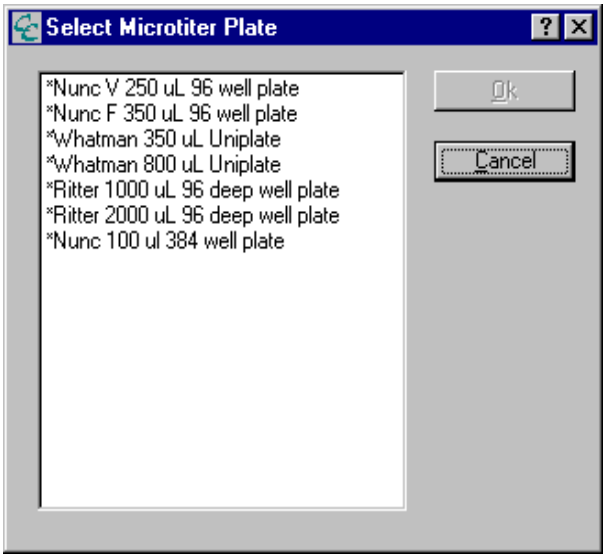
4 Select the desired rack type from the list, then click OK.

CC-Mode checks whether the desired rack fits into the desired position. If you have chosen the wrong rack type, click the right mouse button on the rack and select Remove rack to delete it.

Note that if you are using rack 505H, it must be located next to the dilutor. It cannot be configured in any other position.

5 To specify the type of well plate to be used with racks 505H, 505, 205H, and 205, click the right mouse button on a plate and select Change Microtiter Plate from the pop-up menu.

A list of pre-configured plates appears as shown in the example below. These plates can be used as templates to customize the plates or to create similar plates.


Select Microtiter Plate
*Nunc V 250 uL 96 well plate
*Nunc F 350 uL 96 well plate
*Whatman 350 uL Uniplate
*Whatman 800 uL Uniplate
*Ritter 1000 uL 96 deep well plate
*Ritter 2000 uL 96 deep well plate
*Nunc 100 ul 384 well plate
Ok
Cancel

6 Select the desired plate from the list, then click OK.

7 Use Save As from the File menu to save the modified rack configuration under a new name. Or use Save to overwrite the original configuration.

8 Select Return to Main screen from the File menu to close the Rack Configuration window.

9 If you changed racks as described in steps 3 - 5, specify the new rack configuration as described in *To change the system configuration* on page 21.

New studies will use this Rack configuration. When you return to the main screen as described in Step 8 above, the new configuration is *not* shown at this time.

To design plates

If you want customized microtiter plates that are not in the plate lists, you can enter the dimensions and save the customized sample device with a name of your choice.

CAUTION

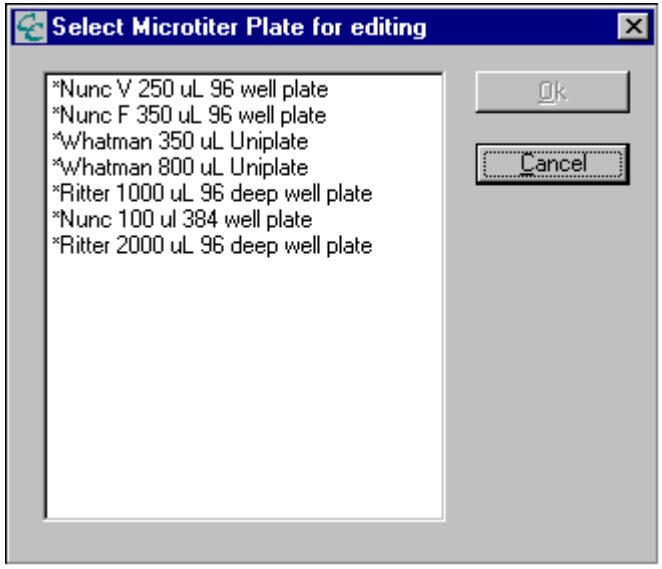
Be careful when designing customized plates. Refer to online help for detailed information. For testing customized plates, loosen the needle to avoid damaging it.

1 **Select Rack Configuration from the Configuration menu in the CC-Mode Main window.**

The Rack Configuration window appears.

2 **Select Edit Microtiter Plate from the Design menu.**

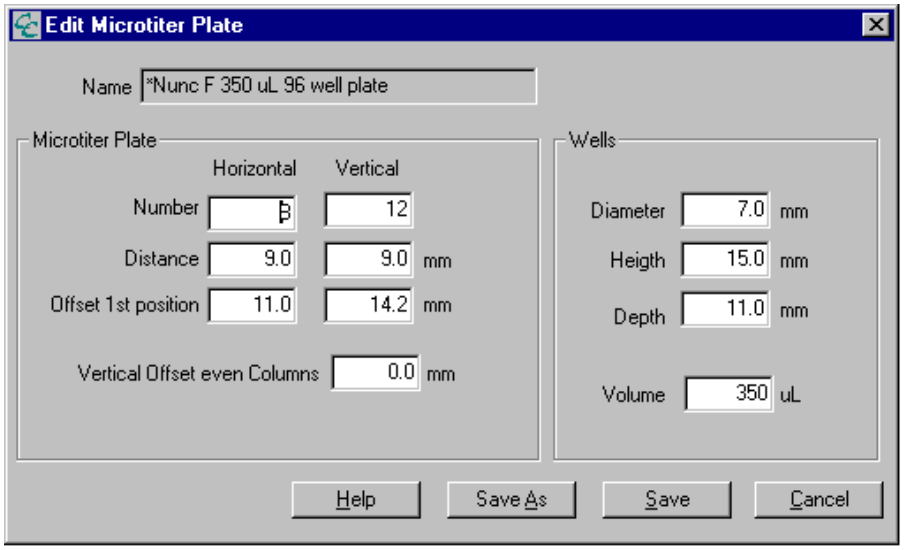
3 **Select the microtiter plate to edit, then click OK.**


Select Microtiter Plate for editing
*Nunc V 250 uL 96 well plate
*Nunc F 350 uL 96 well plate
*Whatman 350 uL Uniplate
*Whatman 800 uL Uniplate
*Ritter 1000 uL 96 deep well plate
*Nunc 100 ul 384 well plate
*Ritter 2000 uL 96 deep well plate
Ok
Cancel

4 **Enter the requested information on the Edit Microtiter Plate dialog box.**

Enter the dimensions to the nearest of tenth of a millimeter. See online help for

more information about the microtiter plate design parameters.



5 Use the Save As button to save the configuration under a new name.

To design racks

If you want customized racks that are not in the rack lists, you can enter the dimensions and save the customized sample device with a name of your choice.

CAUTION

Be careful when designing customized racks. Refer to online help for detailed information. For testing customized racks, loosen the needle to avoid damaging it.

1 **Select Rack Configuration from the Configuration menu in the CC-Mode Main window.**

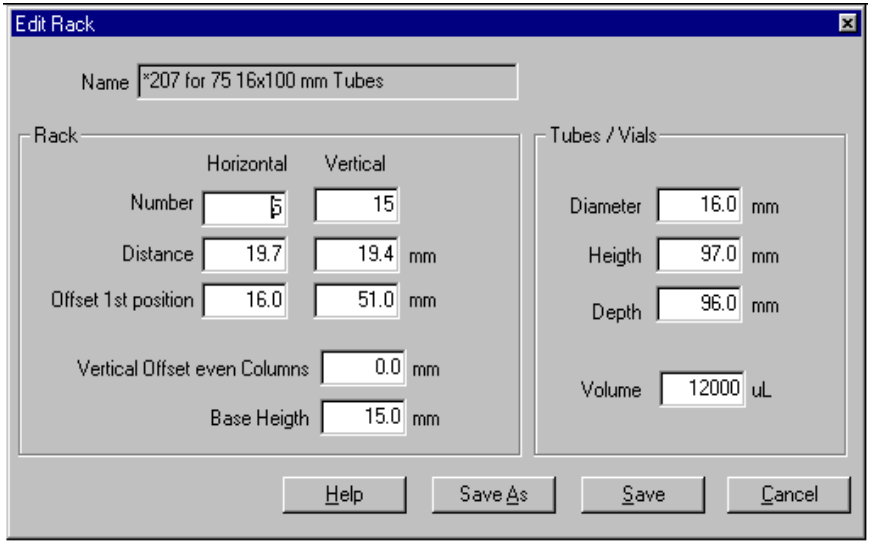
The Rack Configuration window appears.

2 **Select Edit Rack from the Design menu.**

3 **Select the rack to edit, then click OK.**

4 **Enter the requested information.**

Enter the dimensions to the nearest of tenth of a millimeter.



5 **Use the Save As button to save the configuration under a new name.**

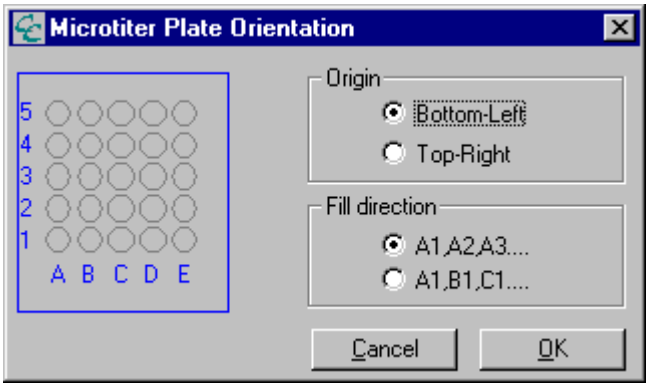
To change plate orientation and fill direction for fraction collection

This procedure applies to the optional Fractionator Mode only.

1 **Select Rack Configuration from the Configuration menu in the CC-Mode Main window.**

The Rack Configuration window appears.

2 **Select Microtiter Plate Orientation from the Options menu.**



3 **Specify the plate orientation as well as the fill direction of the plates.**

The Fill direction specified here is also used to determine the order that samples are added to a study. See chapter 4, “Study Setup” for more information.

3

Sampler Initialization, 32
To flush the syringe and tubing, 34
To test valve switching on the 220 micro plate sampler, 35
To replace the syringe, 36

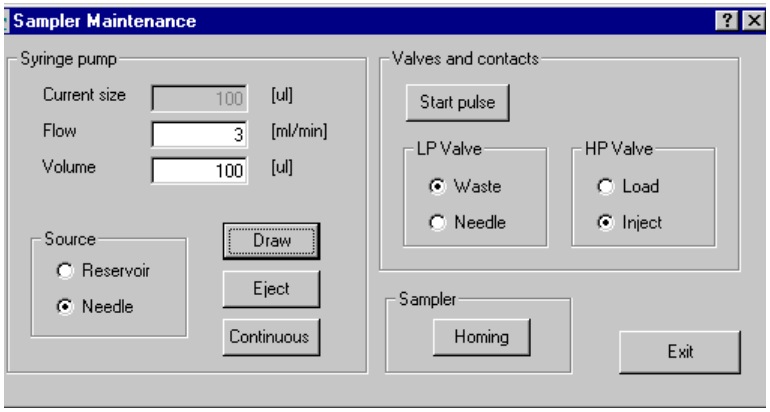
Sampler Initialization and Maintenance

Describes how to initialize and maintain the Agilent 220 micro plate sampler

Sampler Initialization

Use this procedure to initialize the Agilent 220 micro plate sampler.

1 Select Maintenance Mode from the Sampler menu in the CC-Mode Main window to display the following dialog box.



2 Click the Homing button on the Sampler Maintenance dialog box. The following initialization steps will be performed.

- HOME the sampler XYZ system
- Move to the first rinse station
- Setup the correct syringe size and home the syringe piston. Syringe valve was switched already to the needle position
- Switch the LP valve to the WASTE position (fail safe)
- Switch the HP valve to the LOAD position
- Switch initial start relay position (not closed)

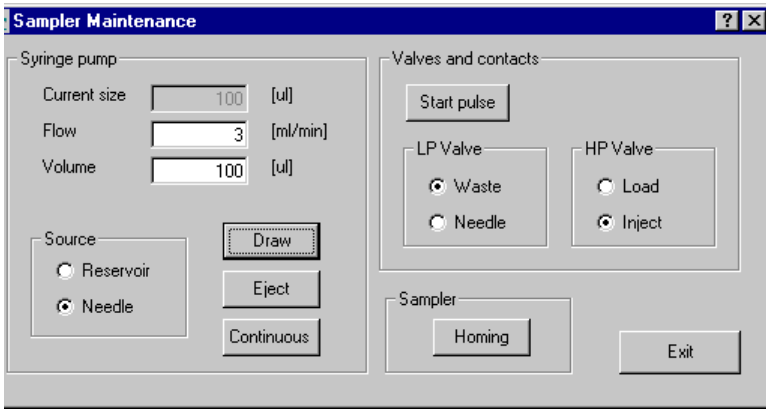
The Homing button moves the sampler to the Home position. The z-drive is activated first, followed by the xy-drive. Finally the proper function of the valves is checked.

This is the same procedure performed when sampler control in CC-Mode is started for the first time.

Pressing the STOP button on the Agilent 220 micro plate sampler, stops the sampler immediately. The arm can be moved freely. During this situation CC-Mode lost control of the sampler and will report an error message. Pressing the START button on the sampler again will initialize the XYZ sampler system. The needle moves to the home position in the rear left corner (not above a rinse station). The complete Agilent 220 micro plate sampler system is NOT initialized until the syringe and valves are also initialized.

To flush the syringe and tubing

1 Select Maintenance Mode from the Sampler menu in the CC-Mode Main window to display the following dialog box.



This dialog box shows the actual status and configuration of the sampler. The field Current size shows the volume of the syringe placed on front of the sampler.

2 Click the Continuous button.

The syringe, tubing and the needle can be primed by using the Continuous button, the button then changes to Stop Pump. The syringe draws liquid from the solvent reservoir and ejects it through the needle to the rinse station.

NOTE The flush volume of the syringe cannot be higher than the volume of the syringe installed and configured in the 'System Configuration' screen (default syringe = 100 µl). The flow can be adjusted depending on the viscosity of the solvent.

NOTE The complete rinsing of the tubing may need some time depending on the syringe size installed and the selected rinse volume and the flow.

The procedure will continue until Stop Pump is clicked.

To test valve switching on the 220 micro plate sampler

1 **Select Maintenance Mode from the Sampler menu in the CC-Mode Main window.**

This dialog box shows the actual status and configuration of the sampler. The current valve positions are shown in the LP Valve and HP Valve fields.

2 **Select a different position for the high pressure (HP) valve and the low pressure (LP) valve.**

The valve position will be changed immediately as follows.

High Pressure valve

The HP valve can be switched at any time. When the Agilent 1100 Series LC is turned on, the position of the HP valve determines the flow path. When the HP valve is in the load position, the pump flow is directed immediately to the detector input. When the HP valve is in the inject position, the sample loop is taken in the flow path as well. The flow path of the detector output is determined by the LP valve position as described above.

The current position of the HP valve can be checked on the sampler itself as follows.

- LOAD ON - valve is in LOAD position (injection through sample loop)
- INJECT ON - valve is in INJECT position (sample loop is switched in the LC flow)

Low Pressure valve

The LP valve can be switched at any time. When the Agilent 1100 Series LC is turned on, the position of the LP valve determines the flow path. When the LP valve is in waste position, the output of the detector flows into the waste container. When the LP valve is in the needle position, the output of the detector flows to the needle. Since the needle is moved over the first rinse station, fluid flows into the rinse station.

The current position of the LP valve can be checked on the sampler itself as follows.

- LED ON - valve is in NEEDLE position
- LED OFF - valve is in WASTE position

To replace the syringe

1 **Select Maintenance Mode from the Sampler menu in the CC-Mode Main window.**

2 **Set the syringe volume to the maximum volume. This enables the syringe to move to its lowest position.**

3 **Click on Draw. The syringe moves.**

4 **At its lowest position the syringe stops automatically.**

5 **Replace the syringe. Take a note of the syringe volume to configure it later in the System Configuration screen.**

6 **Click on Eject. The syringe moves to its home position.**

7 **Exit the Sampler Maintenance screen and configure the new syringe size in the System Configuration screen.**

8 **Exit CC-Mode and restart the sampler.**

The system is now configured with the new syringe size.

4

What is a study, 38
To create a new study, 42
To select samples for the study, 50
To remove samples from the study, 53
To add fractions, 54
To open or edit an existing study, 55
To edit method parameters, 58
To view study lists, 59
To create a partial study, 60
To set up for fraction collection, 61
To set up an FIA study, 64
To report study data and parameters, 66

Study Setup

Describes what a study is and how to set one up, including fraction collection and FIA analysis

What is a study

A study is a series of instructions that automates the analysis, data evaluation and reporting of samples. A study contains at least one analytical method that contains all the parameters for acquisition and evaluation, including integration and reporting. The system can be set up to acquire data from a number of samples by different methods.

A study includes:

- list of samples
- sampler configuration
- analytical and sampler methods
- reporting options
- data file structure

A study might also include:

- list of fractions
- list of expected masses (for INB22002)

Where information is stored

The complete information is stored in a database. Each study is stored under its own name in a separate database. The name has the extension .mdb, for example MyStudy.mdb. Use the Study data path field on the System Configuration screen to specify where to store the studies.

What information is stored

The study database holds the following information:

Plate table

- Plate ID
- Plate type
- Plate directory

Rack configuration

- Rack type
- Rack name
- Microtiter plate orientation
- Fill direction

Study information

- ChemStation method names
- Sample naming
- Subdirectory structure
- Operator name
- Data file path
- Report options

Sample list

- Sample name
- Sample description
- Sample location
- ChemStation method names
- Data file names
- Number of injections

Fraction list

- Fraction location

- Sample name
- Sample location
- Injection number
- Peak number
- Number of fractions
- Start time
- End time

Method-related information

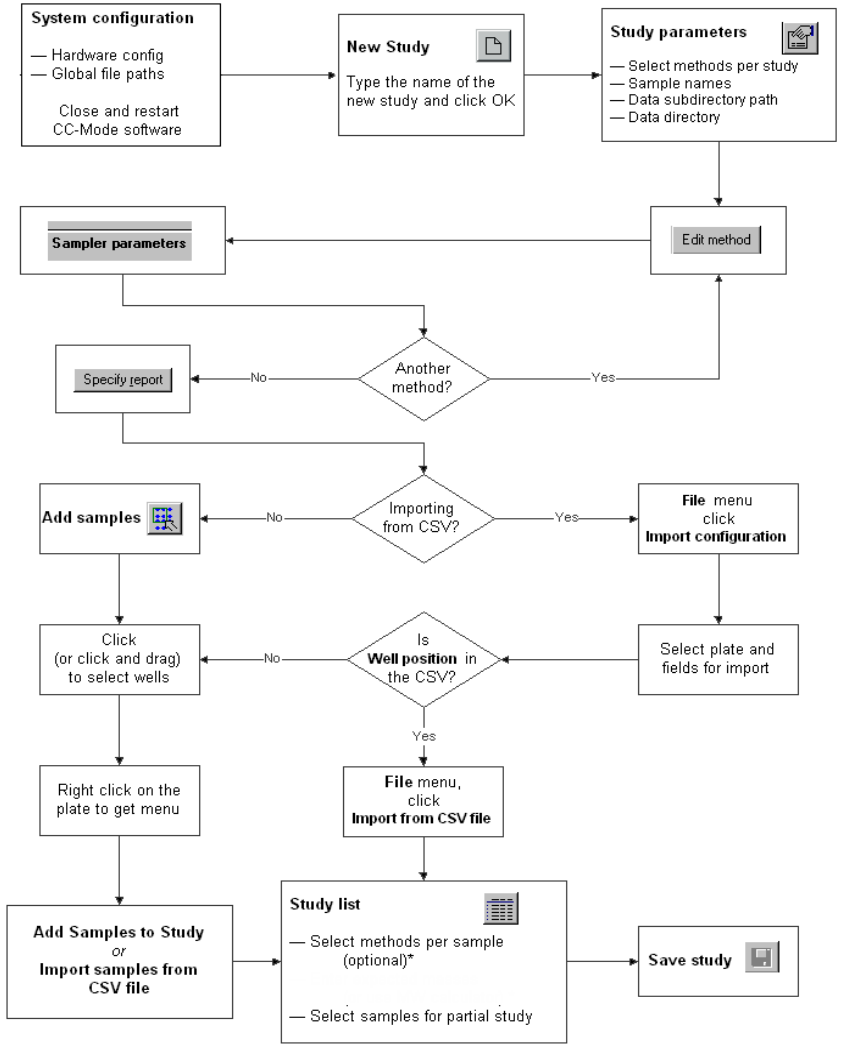
CC-Mode stores method-related information in a file CCMode.mth, which is located in the ChemStation method directory. The CCMode.mth file contains the following information:

- Sampler task list, containing the specific tasks with their parameters
- Purity calculations (optional)

This chapter describes how to set up a study. See chapter 7 to run the study and collect data and fractions.

To set up a new study for INB22000

Note: Some actions will be different if you use the New Study menu item or toolbar button.



* Skip these steps if the information is contained in the imported CSV file.

To create a new study

To create a new study from an exising study by modifying its parameters and saving it under a new name, see *To open or edit an existing study* on page 55.

Otherwise, create the new study as described below.

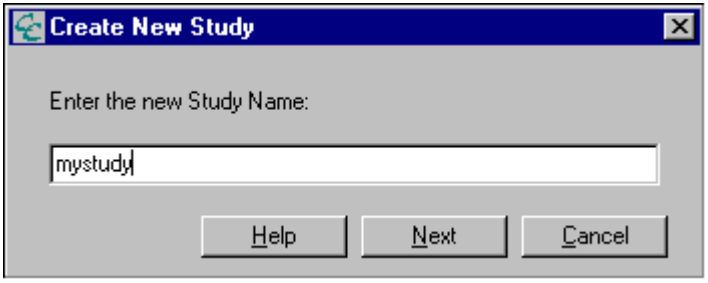
1 Select New Study from the File menu or click the New Study toolbar button shown below.

2 When the Create New Study dialog box appears, read the information, then click Next to continue.

Check the hardware!

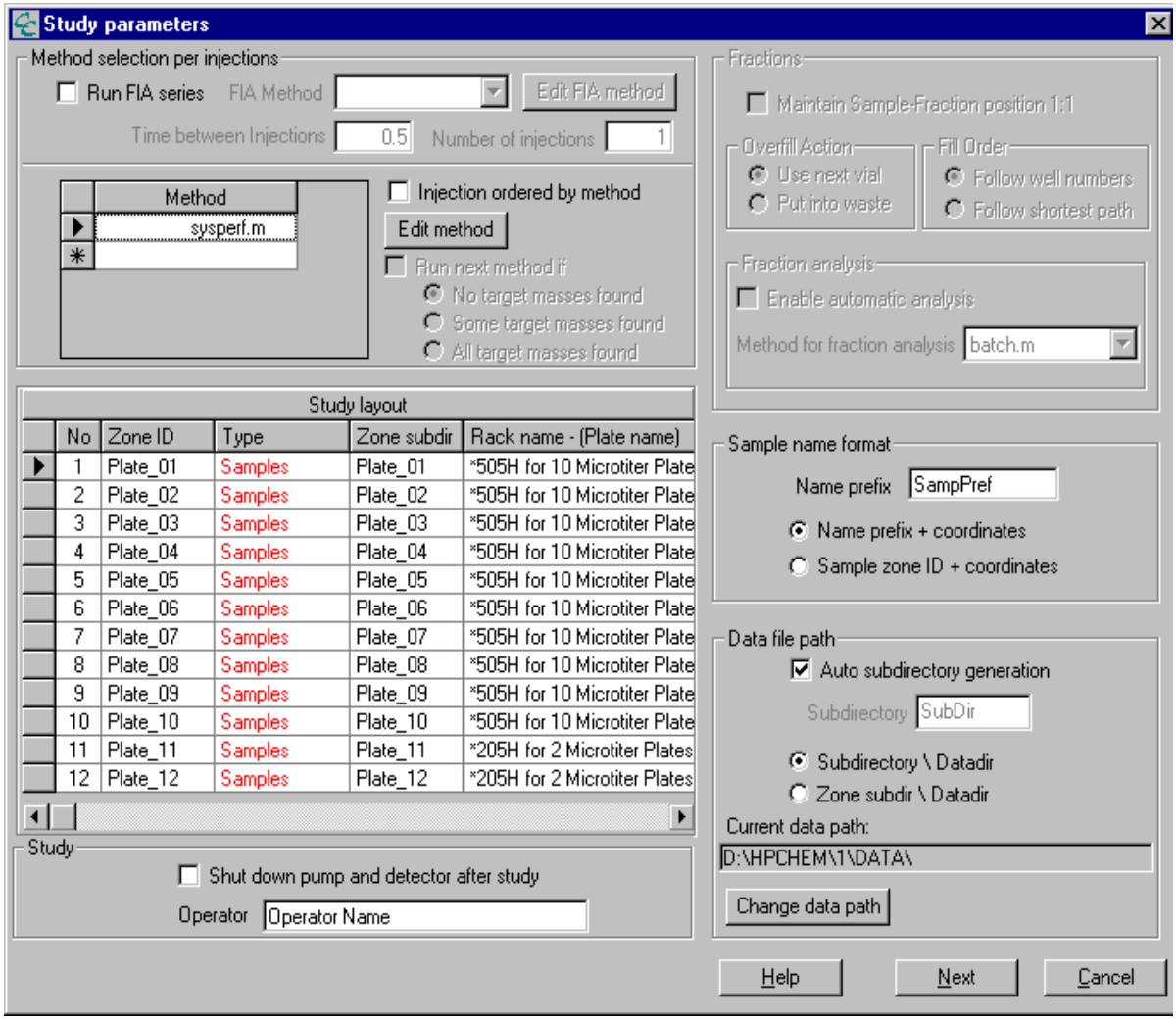
This dialog box gives a summary of the procedure to create a new study. The first step is to make sure by visual inspection that the hardware configuration matches the new study. This must be resolved before going on to the next step.

3 When the following dialog box appears, enter a name for the study, then click Next to continue.



4 When the Study parameters dialog box shown below appears, enter the information in the following pages.

The fields on this dialog box depend on which software options are installed.


Study parameters
Method selection per injections
Run FIA series
FIA Method
Edit FIA method
Time between Injections
0.5
Number of injections
1
Method
sysperf.m
Injection ordered by method
Edit method
Run next method if
No target masses found
Some target masses found
All target masses found
Fractions
Maintain Sample-Fraction position 1:1
Overfill Action
Use next vial
Put into waste
Fill Order
Follow well numbers
Follow shortest path
Fraction analysis
Enable automatic analysis
Method for fraction analysis
batch.m
Study layout
No | Zone ID | Type | Zone subdir | Rack name - (Plate name)
1 Plate_01 Samples Plate_01 *505H for 10 Microtiter Plate
2 Plate_02 Samples Plate_02 *505H for 10 Microtiter Plate
3 Plate_03 Samples Plate_03 *505H for 10 Microtiter Plate
4 Plate_04 Samples Plate_04 *505H for 10 Microtiter Plate
5 Plate_05 Samples Plate_05 *505H for 10 Microtiter Plate
6 Plate_06 Samples Plate_06 *505H for 10 Microtiter Plate
7 Plate_07 Samples Plate_07 *505H for 10 Microtiter Plate
8 Plate_08 Samples Plate_08 *505H for 10 Microtiter Plate
9 Plate_09 Samples Plate_09 *505H for 10 Microtiter Plate
10 Plate_10 Samples Plate_10 *505H for 10 Microtiter Plate
11 Plate_11 Samples Plate_11 *205H for 2 Microtiter Plates
12 Plate_12 Samples Plate_12 *205H for 2 Microtiter Plates
Sample name format
Name prefix
SampPref
Name prefix + coordinates
Sample zone ID + coordinates
Data file path
Auto subdirectory generation
Subdirectory
SubDir
Subdirectory \ Datadir
Zone subdir \ Datadir
Current data path:
D:\HPCHEM\1\DATA\
Change data path
Study
Shut down pump and detector after study
Operator
Operator Name
Help
Next
Cancel

Method selection per injections

Run FIA series

Use this option as described in *To set up an FIA study* on page 64 if you want to use flow injection analysis.

Injection ordered by method

When this option is *not* selected, each sample is analyzed with all the methods specified for it. Then the next sample is analyzed with all the methods specified for it, and so on. The order that the samples are injected is the same as the order in which they were added to the study, as described in *To select samples for the study* on page 50.

This option can only be selected when the study does not yet contain any samples. When this option *is* selected, the injections are sorted by method. The order of the methods is determined by the method list in the Study Parameters screen. All samples using the same method are injected first, then the next method is loaded and all samples using this method are injected and so on. This minimizes the loading of methods and thus reduces the time between injections. The order that samples with the same method are injected is determined by the order in which the samples are added to the study. When you select the Injection ordered by method option, all methods specified for each sample are run unconditionally. See online help for more information.

Run next method if

See *To set up study parameters - LC/MSD* on page 95 for LC/MSD.

To add a method

Each method represents one injection. If for example you wish to inject the sample twice with the same method, the method name should appear twice in the list. These method names will be used for all subsequent samples added to the study. The method names for existing samples will not change, unless you remove them and add them again to the study.

Ü **Click on blank cell for Method in the line marked with an asterisk (*) in the left column. A list of available ChemStation methods will be displayed.**

Ü **Click on a method name to add the method to the list.**

Study layout

This section of the screen shows a list with the plates and racks that are configured with the Rack Configuration, starting with plate number.

Zone ID

These values can either be typed in or entered using a barcode reader placed between the keyboard and PC. The Zone ID also appears below the plates in the CC-Mode Main window. The Zone ID is used for sample naming; see *Sample name format* on page 45 for more information.

Type

Click on cell in the Type column to display a pop-up menu. Select a type for the plate or rack from the choices shown below:

Samples - these plates or racks are reserved to hold the samples. Labels for sample plates or racks are red.
Fractions - these plates or racks are reserved to hold the fractions collected during the study. Labels for fraction plates or racks are blue.
Preparation - these plates are reserved for sample prep use.

Zone subdir

The subdirectory is used to store the data files in separate directories per plate, if the Auto subdirectory generation option is *not* selected in the Data file path section of the screen.

Rack name – (Plate name)

Shows the name of the sampler rack, with the plate name in parentheses.

Study

Shut down pump and detector after study

Select this option to shut down the instrument after the last run.

Operator

Enter the name of the operator. The name entered here appears on the ChemStation report.

Fractions

See *To set up for fraction collection* on page 61 for a description of the Fraction options on the Study parameters screen.

Sample name format

The sample name is a character string from 1 to 16 characters long, formed in one of the following ways:

Name prefix + coordinates

The complete sample name is formed by the prefix + the sample coordinate. For example, Samppref03A01, where Samppref is the prefix and 02A01 the coordinate. Since the coordinate could be up to 5 characters long, the prefix length must not exceed 11 characters.

Sample Zone ID + coordinates

The complete sample name is formed by the Zone ID + the sample coordinate. For example, Zone_1_A02, where Zone_1_ is the Zone ID, entered in the Study layout table and A02 is the sample coordinate within the plate. Since the coordinate could

be up to 3 characters long, the Zone ID length must not exceed 13 characters.

Data file path

These parameters control the destination of your ChemStation data files.

Subdirectory \ DataDir

The data files are placed directly under the subdirectory specified by the user. When the Auto subdirectory generation option is selected, the subdirectory name is automatically generated as yyyymmdd.nnn, where:

yyyy is the 4 digits of the year
mm is the month
dd is the day
nnn is a number starting at 1 and incremented each time a subdirectory is created on the same date.

When the Auto subdirectory generation option is *not* selected, the specified Subdirectory is used for the subdirectory name. The Subdirectory name must be a valid file name and not exceed 8 characters.

Zone subdir \ Datadir

When this option is selected, a subdirectory is created for each zone (plate). The data files are placed in the Zone subdirectories specified in the Study layout table. See *Study layout* on page 44 for more information.

Change data path button

Click this button if you want to specify a different data path to store the subdirectories and data files. Otherwise the data path specified on the System Configuration screen is used as described in chapter 2.

5 When you have entered all the Study parameters, click Next to continue.

6 When the Specify Report dialog box shown on the next page appears, enter the information as described below.

The Specify Report dialog box lets you define the reports generated by CC-Mode.

Repo rting

Enable ChemStation data analysis report

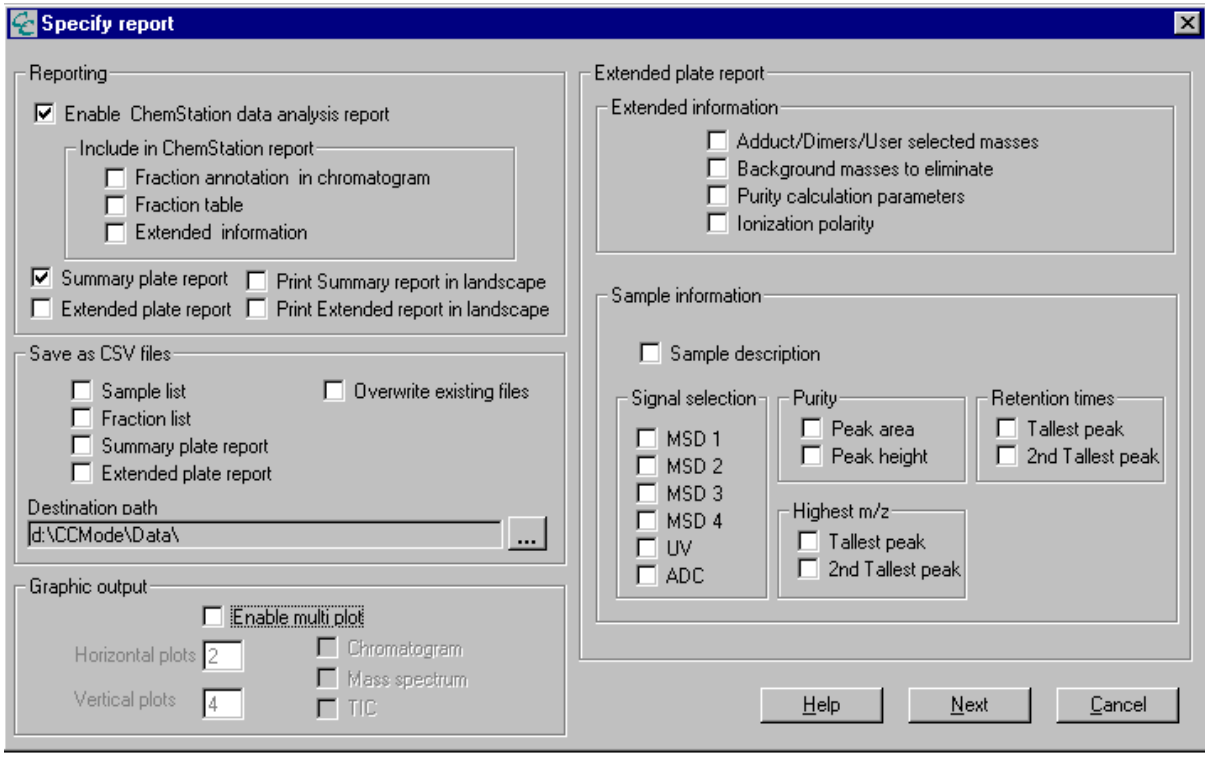
When this option is selected, the reports specified in the ChemStation method are generated after each injection. The destination for the report is specified in the ChemStation method. When this option is *not* selected, all the reports produced by ChemStation are suppressed.

Fraction annotation in chromatogram

When selected, the chromatogram produced by ChemStation will be enhanced with the fraction information.

Fraction table When selected, a section containing fraction data is included in the ChemStation report.

Extended information See *To specify the report format - LC/MSD* on page 100 for LC/MSD reporting options. Depends on which products are installed.



Save as CSV files

You can generate CSV files for the following data:

- Sample list
- Fraction list
- Summary plate report
- Extended plate report

Destination path

Displays the location where the CSV files will be stored. Click the browse button (...) to select a different location for the CSV files. The location where the CSV files are stored is defined as follows: <Destination path>\<Study name>\CSV\.

The CSV files have the following names:

- Sample.CSV
- Fraction.CSV
- Sum_xx.CSV, where xx is the plate number
- Ext_xx.CSV, where xx is the plate number

Overwrite existing files

Select this option to overwrite CSV files of the same name in the same directory. When this option is not selected, the CSV directory name will have a counter appended to it, which is incremented each time new files are created as follows:

<Destination path>\<Study name>\ CSVxxx\<csvfiles>

where xxx is a number starting at 000 and incremented by one each time new files are created. When the number reaches 999, no more files are created.

Graphic output

Enable multi plot
Horizontal plots
Vertical plots

When selected, multiple plots can appear on one page. The number of plots per page is determined by the Horizontal plots and Vertical plots parameters. For example, if Horizontal plots is set to 3 and Vertical plots is set to 4, 12 plots are printed per page. The number of plots per sample depends on which options have been selected. Plots from different samples are printed continuously.

Chromatogram

When selected, the chromatograms are printed on the report.

Mass spectrum/TIC

See *To specify the report format - LC/MSD* on page 100 for LC/MSD reporting options. Depends on which products are installed.

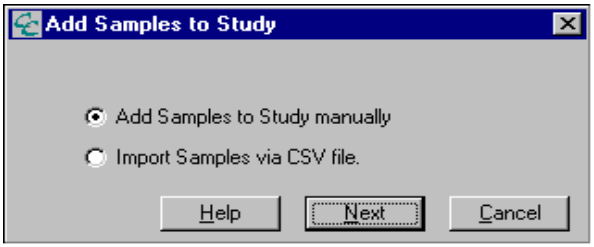
Extended plate report

Extended information

See *To specify the report format - LC/MSD* on page 100 for LC/MSD reporting options. Depends on which products are installed.

7 When you have entered all the report parameters, click Next to continue.

8 **When the Add Samples dialog box appears, select the way you want to add samples to the study. Click Next to continue.**



If you select the Add Samples to Study manually option, an information-only dialog box appears. Read the information, then click **Next** to continue.

9 **Add samples to the study as described in *Adding samples to a study manually* on page 50 or *Importing sample data from another application* on page 51, depending on which option you selected above.**

10 **When you have finished adding the samples to the study, click the Save Study toolbar button, or select Save Study from the File menu.**

To select samples for the study

Samples can be added to a study either manually or by importing the information from another application via .CSV file as described below.

Adding samples to a study manually

If you are adding samples as part of the New Study task described in the previous section, skip to step 3.

1 Verify that the Add Samples button in the toolbar shown below is selected.

The Status Bar shows “Add Samples” when this mode is selected.

2 Check the settings in the Study Parameters screen. The following parameters will be used for samples added to the study.

- Sample Name format
- ChemStation Method(s)

3 Select samples in the following ways.

NOTE

Select the samples in the order you want to inject them. When the study is run, the samples are injected in the order they are added to the study.

- Randomly - by clicking on individual sample wells
- Dragging - by holding down the left mouse button and drawing the rectangle over the wells
- Row - by clicking on the numbers on the left side of the plate to select an entire row
- Column - by clicking on the letters below the plateto select an entire column

4 After you have selected one or more samples, right-click on one of the plates.

5 Select Add Samples to Study from the pop-up menu.

The selected samples will be added to the sample list in the study in the order of their selection. Sample selection can be verified in two ways:

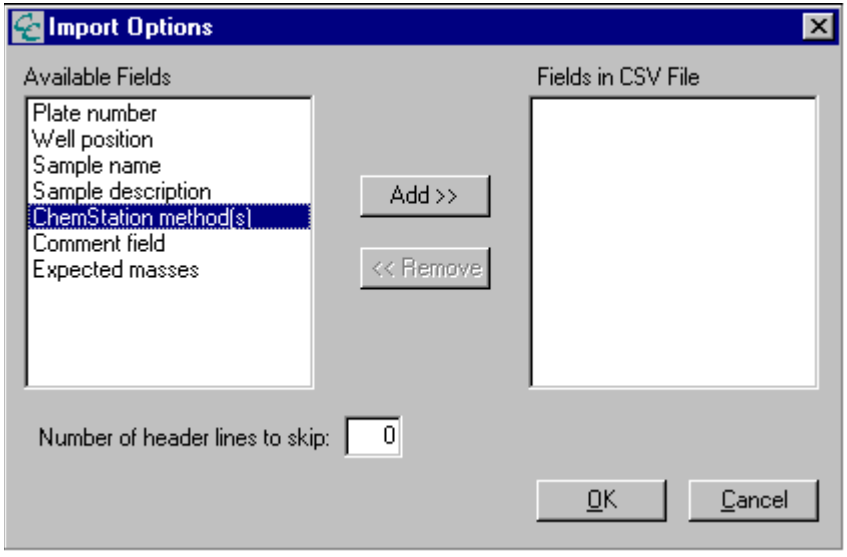
- Samples in the study are red colored.
- Selected samples are added to the Study List.

Samples are added with the current method selected in the Study Parameters screen. If you change the method in the Study Parameters screen, previously added samples in the study list are *not* affected.

Importing sample data from another application

You can import sample data from external applications like Microsoft Excel (file extension .CSV) to create study lists as described below. If you are importing samples as part of the New Study task described in the previous section, these steps will appear in a slightly different order.

1 Select Configure CSV file layout from the File menu to open the Import Options dialog box.



2 Select the fields to import.

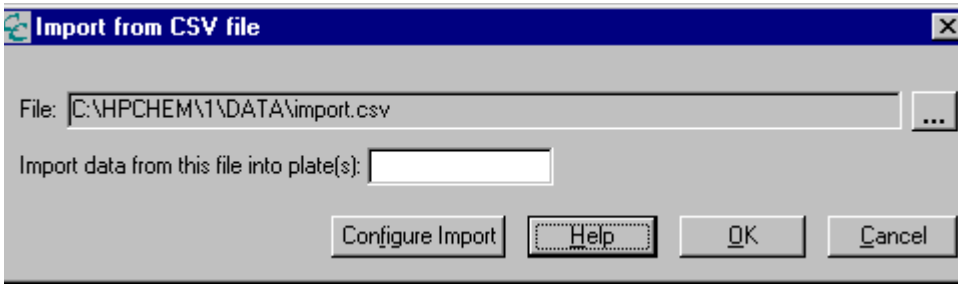
The relative order in the CSV file Comment field is used to tell the software which

fields to import.

3 Enter the number of header lines.

4 Click OK.

5 Select Import from CSV File from the File menu.



If the Import configuration layout specifies plate numbers, then the Import data from this file into plate number option is not available.

Otherwise, you can specify the plate/rack you to use to import data. Multiple plates can be specified at once, using the following format:

- Single plates separated by commas, for example: 1,3,5,6
- Range of plates for example 3-6, which means plates 3,4,5,6.
- Or combinations of both like 2,4-6, which means plates 2,4,5,6.

When using multiple plates, each plate will contain the same samples, as specified by the CSV file. The plates must be of the same type.

NOTE List the samples in the CSV file in the order you want to inject them. When the study is run, the samples are injected in the order they appear in the CSV file.

6 Accept the displayed CSV file or use the browse button (...) to find the desired file.

The default path and file name for the csv file are specified in the System Configuration dialog box described in chapter 2.

To remove samples from the study

Use the following steps if you want to remove any of the previously selected samples from the study.

1 **Select the samples you want to remove from the study as described earlier in this section.**

2 **After you have selected one ore more samples, right-click on one of the plates.**

3 **Select Remove Samples from Study from the pop-up menu.**

To add fractions

It is possible to inject collected samples from fraction plates as follows:

1 **Click with the left mouse button on the desired green fraction well.**
A white rim will appear around the fraction well to indicate it has been selected.

2 **Repeat this for each fraction you want to inject.**

3 **After you have selected one or more fractions, right-click on one of the fraction plates.**

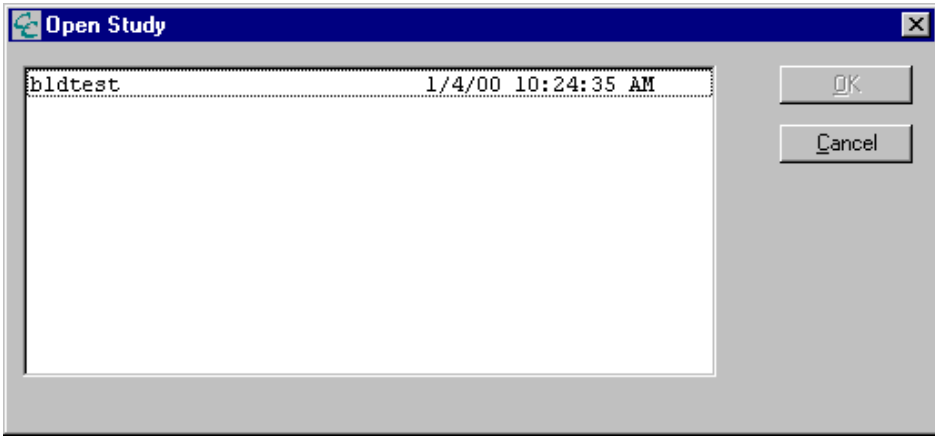
4 **Select Mark Fraction for Analyze from the pop-up menu.**

The fractions that have been added to the study will turn blue.
The Study List shows the fractions that will be injected when the study is run.

To open or edit an existing study

The name of the current study is displayed in the title bar of the CC-Mode Main window. To edit the current study, skip to step 3.

1 To open a different study to edit, select Open Study from the File menu to display the dialog box shown below.



2 Click on the name of the study you want to edit in the list, then click OK.

3 **Click the Study Parameters toolbar button.**

The Study Parameters dialog box allows you to set the following parameters necessary to analyze samples and generate reports.

- ChemStation method selection. See *To select methods* on page 57.
- 220 Sampler tasks (described in chapter 5)
- Purity calculations. See *To set up purity calculations* on page 97.
- Study layout. See *Study layout* on page 44.
- Sample name format. See *Sample name format* on page 45.
- Data file path. See *Data file path* on page 46.
- Report options (click the Specify report button). See *Repo rting* on page 46.

4 After making the desired changes, save the modified study.

Ü **If you don't want to overwrite the original study, select Save Study As from the File menu and enter a new name.**

Ü **Otherwise, select Save to replace the original study.**

To select methods

Ü **On the Study Parameters screen, select the names of one or more ChemStation methods you want to use for data acquisition and processing.**

Each method represents one injection. If for example you wish to inject the sample twice with the same method. the method name should appear twice in the list. These method names will be used for all subsequent samples added to the study. The method names for existing samples will not change, unless you remove them and add them again to the study.

For more information about options for running methods, see *Method selection per injections* on page 43. See *To set up study parameters - LC/MSD* on page 95 for method-related study options LC/MSD analyses,

To add a method

1 **Click on the blank cell for Method in the line marked with an asterisk (*) in the left column.**

A list of available ChemStation methods appears.

2 **Click on a method name to add the method to the list.**

To replace a method

1 **Click on the name of the method you want to replace.**

2 **Select a new method from the drop down list.**

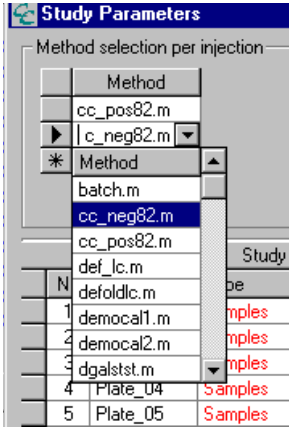
To delete a method

1 **Select a method by clicking in the left- most column next to the method name.**

2 **Press the Delete key from the keyboard.**

To edit method parameters

1 **Select a method by clicking in the box left to the method name.**



2 **Click the Edit Method button to display the Edit Method screen.**

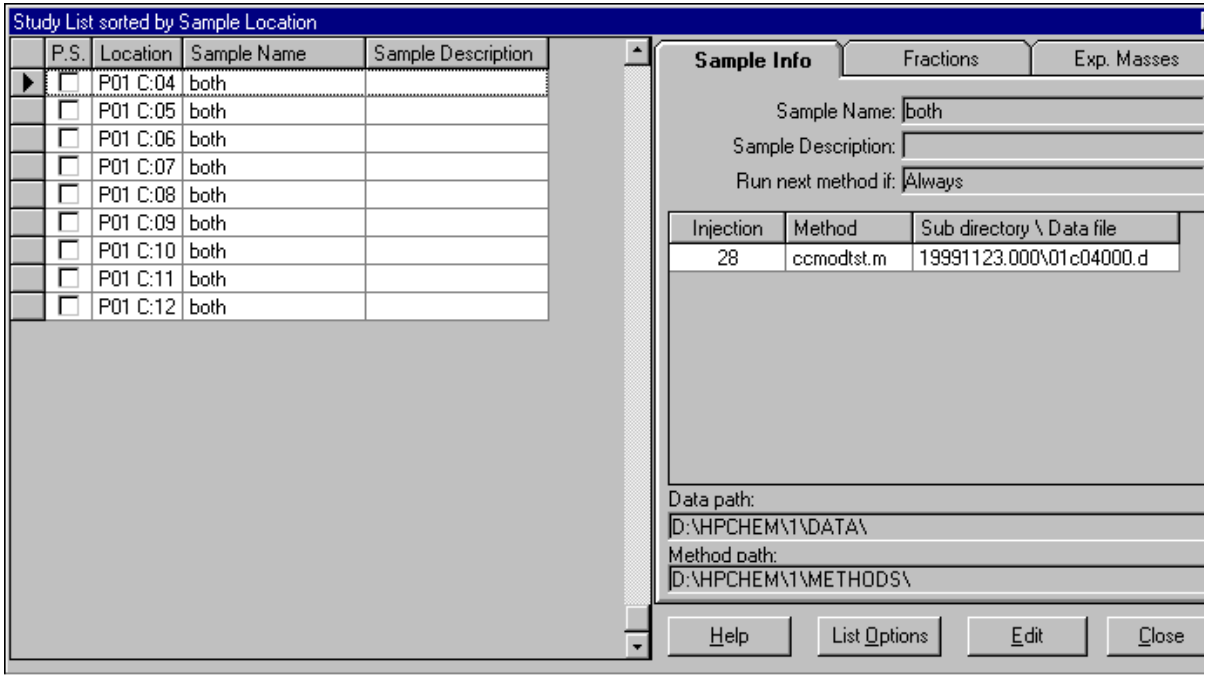
3 **See chapter 5 for information on setting the sampler parameters.**

NOTE

Saving of a Chemstation method should be avoided while a study is running. Loss of data could result.

To view study lists

1 **Click the Study List button from the toolbar.**



2 **If you want the samples to appear in a different order in the Study List, click the List Options button and select one of the following options.**

- Sort list by Injections
- Sort list by Sample Location
- Show only samples in Partial Study
- Show Analyze Fractions List

3 **Click OK.**

The samples will be listed in the selected order in the Study List dialog box.

To create a partial study

Create a partial study when you want to analyze only some samples in your study.

Interactively (from the Study Layout screen)

1 **Verify that the Select button in the toolbar shown below is selected.**

The Status Bar shows “Add Samples” when this mode is selected.

2 **Select one or more samples as described earlier in this chapter.**

3 **After you have selected the samples, right-click on one of the plates.**

4 **Select Add Samples to Partial Study from the pop-up menu.**

5 **Right-click on one of the plates, then select Show Samples in Partial Study from the pop-up menu.**

When this menu item is selected, a check mark appears in front of the menu item and the samples in the partial study are marked with a cross (+) inside the sample wells on the display.

Through a study list

This is the only way to modify a partial study in INB22004 Standalone Data Analysis.

1 **Open the study list by clicking on the Study lists toolbar button.**

2 **If not already selected, select the Sorted by Sample Location view using the List Options button on the Study List dialog box.**

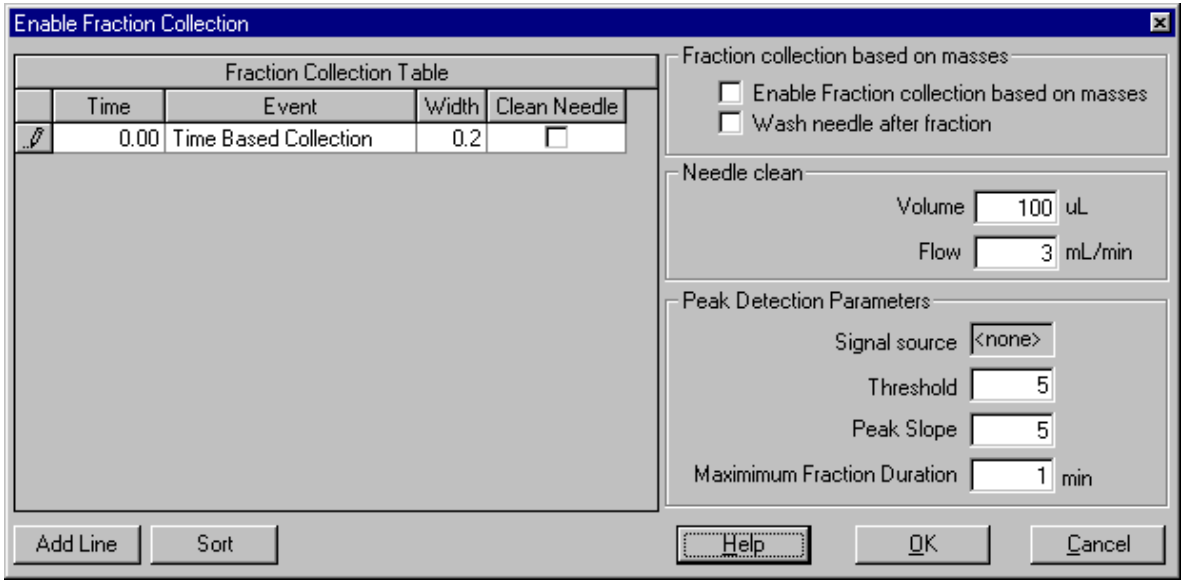
3 **Click in the P.S. column next to each sample that you want to include in the partial study.**

To set up for fraction collection

1 **Select the Edit Method button from the toolbar in the CC-Mode Main window (or click the Edit Method button from the Study Parameters dialog box).**

2 **Select Enable Fraction Collection from the Sampler Parameters task list, then click the Add button.**

3 **Click the Edit button to display the Enable Fraction Collection dialog box shown below.**



4 **Set the desired fraction collection options, then click OK.**

See online help or the Sampler tasks reference in chapter 5 for information on various fraction collection options.

5 **Set the fraction options on the Study parameters screen as described below.**

Type

In the Study layout portion of the screen, click on a cell in the Type column, then select **Fractions** from the pop-up menu. These selected plates or racks are reserved

to hold the fractions collected during the study. Labels for fraction plates or racks are blue.

Maintain Sample Fraction Position 1:1

When this option is selected, the system collects the fractions in the corresponding well position as the sample. For example, samples are in plate P1. Plate P7 is designated as fraction plate. A fraction originated from a sample in well P1 C:03 will be collected in fraction plate P7 C:03. Fraction from sample P1 C:04 will be collected in fraction plate P7 C:04.

This option implies the following behavior and restrictions:

1 Only one fraction well per sample can be used, i.e. only one fraction can be collected per sample.
2 The Overfill Action is automatically set to Put into waste.
3 The shape of sample and fraction plates must be the same, i.e. they must have the same number of rows and columns.
4 There must be a fraction plates for every sample plate.

For information on using this option to purify the component in the fraction, see *Pooling* on page 115.

Overfill Action

The Overfill Action determines what happens when a fraction well becomes full during collection.

Use next vial - the fraction will continue in the next free vial.
Put into waste - the remainder of the fraction will go to waste; i.e. will *not* be collected as a separate fraction.

Fill Order

The Fill Order determines the order in which the fraction wells are filled.

Follow well numbers - the fraction wells are filled in the order given by the fraction numbering. For example: fractions are filled starting at A01 then A02, A03 up to A12, continuing at position B01, B02 B03.... B12, etc. Or A01, B01, C01 .... H01 then A02, B02, C02 .... H02.

Follow shortest path - when moving from one well to the next, the shortest distance between wells on the same plate is used. For example, starting at A01 then A02, A03 …… A12 then B12 ..... B03, B02, B01 then C01 etc. Or A01, B01, C01 .... H01 then H02, G02, F02 .... A02.

Fraction analysis

Select the Enable automatic analysis option to inject all collected fractions automatically at the end of the study. The fractions are analyzed using the method selected in the Method for fraction analysis list, which shows the available

ChemStation methods. The same method as used for fraction collecting cannot be used. The selected method must *not* contain the Enable Fraction Collection sampler task.

See Also

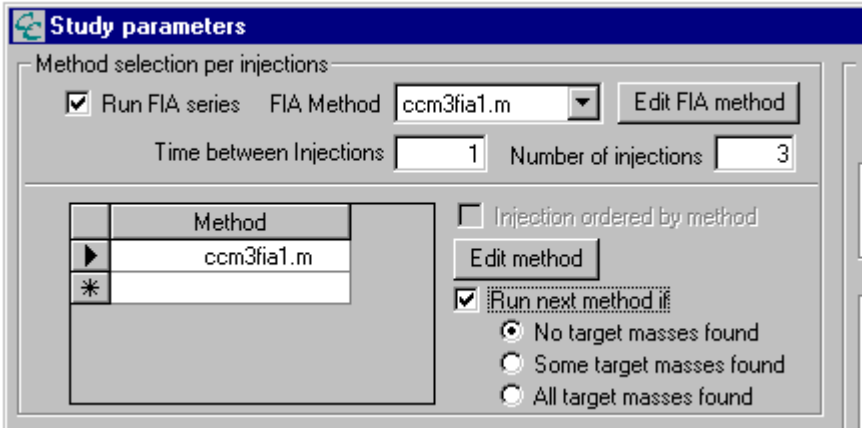
See *To set up for fraction collection - LC/MSD* on page 109 for mass-based fraction collection options.

To set up an FIA study

Flow Injection Analysis (FIA) allows you to make a series of injections quickly. Normally the injections are done without a chromatographic column, but can be done with chromatography under isocratic conditions. The data for all injections is stored in a single data file. To set up an FIA study:

1 Open the CC-Mode Study parameters and select the Run FIA series option as shown below.

The fields on this dialog box depend on which products are installed.



When the study already contains samples, you cannot select or deselect Run FIA series. In that case, remove existing samples from the study, then set this option.

2 Select the ChemStation method to use for FIA.

The ChemStation method must have set the Enable FIA series in method flag. CC-Mode checks for the state of this flag. The Time between Injections and the FIA table in the ChemStation method are not of importance, since CC-Mode overrides these settings.

3 Set the time between injections.

This is the time in minutes from one injection to the next. When the system injects the first sample, it measures the minimum time needed by the sampler to perform the tasks, specified in the method. The minimum time between injections that can be specified is 0.4 minutes.

If this task-time is longer than the time value specified in Time between Injections, the system updates this entry with the minimum time needed to perform the sampler tasks. In order to speed up the time between injections, minimize the number of sampler tasks in the method.

4 Set the number of injections.

This value defines the number of injections per FIA and thus per datafile.

5 Click the Edit FIA method button to display the Edit Method dialog box.

Sampler parameters

The Enable Fraction Collection task is not allowed in the Sampler task list for FIA methods.

Purity calculations

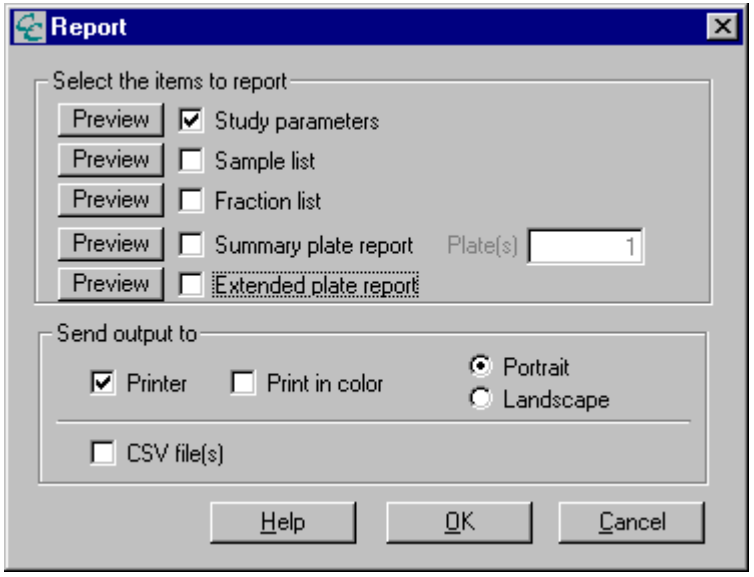
Select the Enable purity calculation and Flow injection (Ion abundance) options.

See Also

Chapter 7 to acquire data for the study.

To report study data and parameters

1 Select Reporting from the File menu or click the Print toolbar button.



2 Select Study parameters and an output destination, then click OK.

The items you select will be sent to the specified destination.

See online help for detailed information about other options.

5

To set sampler parameters, 68
Sampler tasks reference, 70
    Create Air Gap, 70
    Draw Sample, 71
    Inject Sample, 71
    Centered Loop Fill (called Partial Loop Fill in the previous release), 72
    Partial Loop Fill and Inject, 74
    Rinse Needle Inside / Needle Outside / Injection Port, 75
    Wash Vial, 75
    Sample Preparation, 76
    Draw from Destination, 78
    Enable Fraction Collection, 78
    Enable Fraction Collection Delay Sensor, 83
Examples of sampler task lists, 84
To optimize sampler performance, 88

Sampler Tasks Setup

Describes how to set parameters on the Agilent 220 Micro Plate Sampler

To set sampler parameters

1 **Select the Edit Method button from the toolbar in the CC-Mode Main window (or click the Edit Method button from the Study Parameters dialog box).**

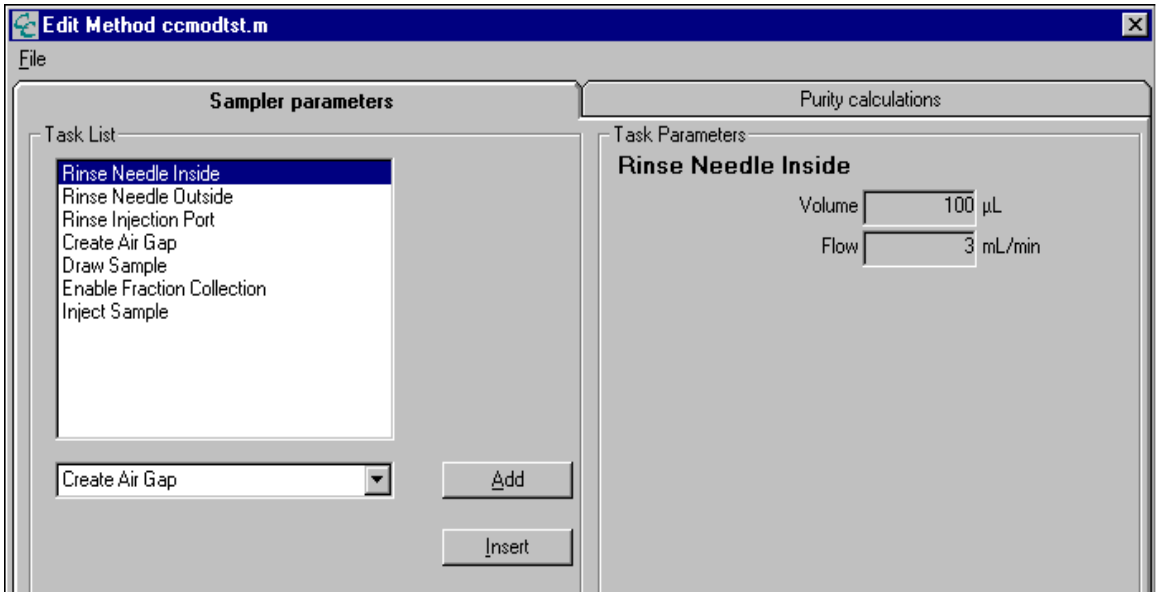
2 **Click the Sampler parameters tab.**

3 **From the Task List, select the tasks to be used for sample analysis and enter the required parameters for each task.**

The tasks will be executed in the order they appear in the Task List. See *Sampler tasks reference* on page 70 for more information on individual sampler tasks.

4 **Click OK.**

The sampler tasks are added to the ChemStation method and are stored in ChemStation method directories.



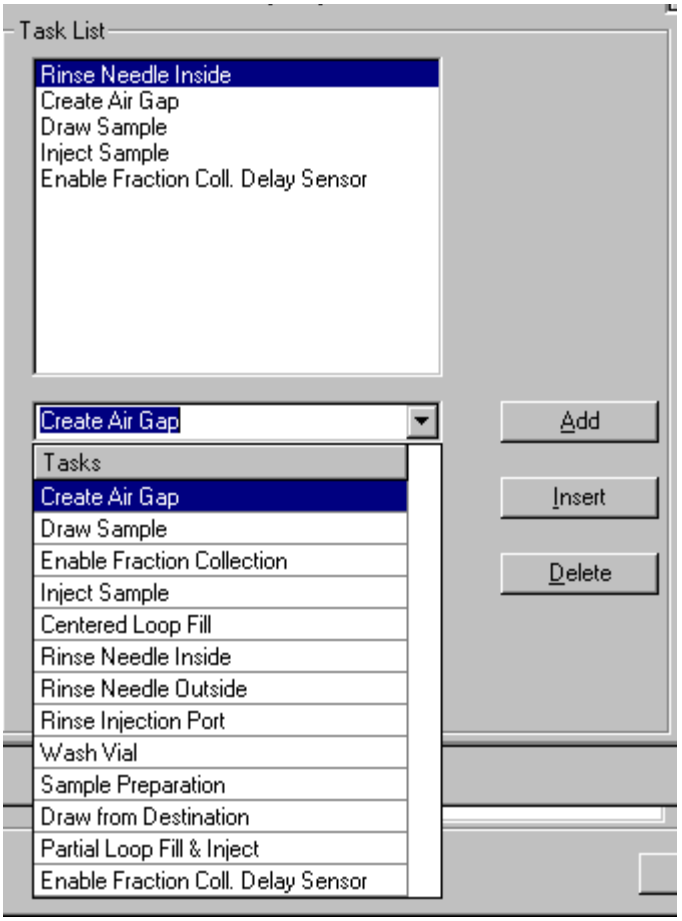
CAUTION

ChemStation method parameters, such as data acquisition and data analysis parameters, cannot be modified or saved using CC-Mode software. These parameters can only be edited and saved using the ChemStation software.

5 If more than one ChemStation method is selected, click on the next method in the Method list and proceed as described in steps 1-4 above.

All selected ChemStation methods will now be used for all subsequent samples selected in the main screen for analysis.

Sampler tasks reference



Create Air Gap

The Create Air Gap task does the following:

1 Raises the needle to its highest position.
2 Rotates the syringe valve to the needle position.
3 Draws a specified volume of air into the needle using a specified flow rate.

To keep liquids separated in the needle/tubing, you should include an air gap before drawing the sample. It is recommended that an air gap volume of about 10 mm in length should be generated inside the transfer tubing. The following air gap volumes are recommended for different tubing diameters:

- 2 µl for 0.4 mm ID tubing
- 3 µl for 0.5 mm ID tubing
- 6 µl for 0.8 mm ID tubing
- 38 µl for 2.0 mm ID tubing

Draw Sample

The specified sample volume is drawn from the sample device. The maximum sample volume depends on the syringe size and the volume of the air gap which separates the sample from the liquid in the needle/tubing. The tasks Draw Sample and Inject Sample used together perform a standard injection. See *Inject Sample* on page 71.

CAUTION The total volume (sample and air gaps) cannot be higher than the syringe volume.

The Draw Sample task does the following:

1 Rotates the syringe pump's valve to the needle position.
2 Moves the needle and lowers it into the selected sample device.
3 Draws the selected volume of sample at a specified flow rate.
4 Moves the needle to the home position.
5 A needle offset can be selected which allows the drawing of a sample from a defined vial level.

Inject Sample

The tasks Draw Sample and Inject Sample used together perform a standard injection. See *Draw Sample* on page 71.

The Inject Sample task does the following:

1 Moves the needle into the injection port.
2 Rotates the injection valve to the load position.
3 Ejects the injection volume into the sample loop.

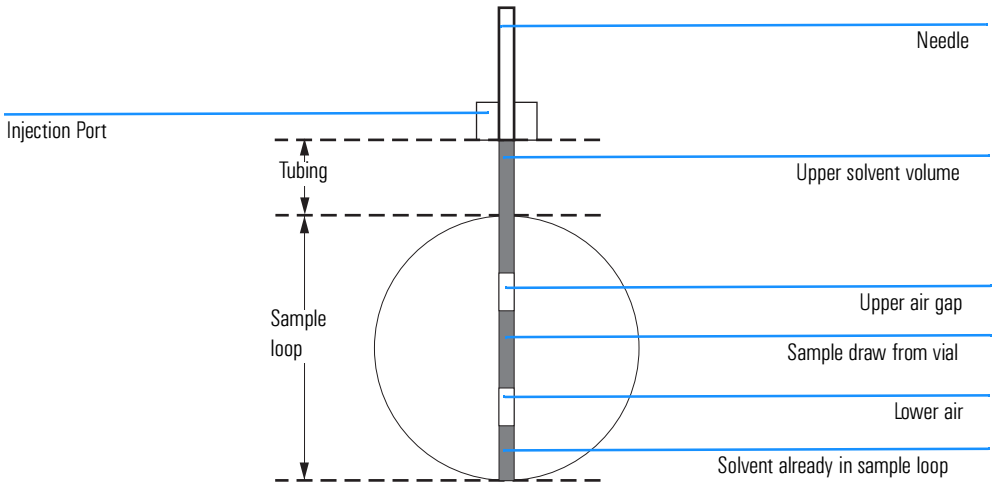
4 Waits for a specified equilibration time.
5 Rotates the injection valve to the inject position to inject the injected volume onto the LC column.

Centered Loop Fill (called Partial Loop Fill in the previous release)

The Centered Loop Fill task is used to partially fill the loop and does the following:

1. Draws the specified Upper Solvent Volume from the solvent container.
2. Draws an upper air gap with the specified volume.
3. Draws the sample with the specified Sample Volume and Flow.
4. Draws a lower air gap with the specified volume.

The Centered Loop Fill screen lets you enter parameters for the centered loop fill and calculates the correct value for the upper solvent volume.



Calibrated tubing volume

Enter the volume of the tubing that connects the injection port to the injection valve. The tubing installed is 4 µl. If you change the size of the tubing, determine the volume.

Sample Loop volume

Calculate volume
The volume is calculated using the values entered for Sample loop length and inner diameter (see below). The calculated volume is displayed in the Volume field after the Calculate button is clicked,

Enter volume
When the volume of the sample is known, choose this option and enter the volume directly in the Volume field (see below).

Sample loop length — Enter the length in millimeters of the connected sample loop tubing. Used to calculate the sample loop volume.

Sample loop inner diameter — Enter the inner diameter in millimeters of the connected sample loop tubing. Used to calculate the sample loop volume.

Volume — Enter the volume of the sample loop in milliliters.

Sample volume — Enter the volume of sample to inject. The sum of the sample volume + upper air gap + lower air gap cannot be greater than the calculated loop volume.

Sample draw flow — Enter the flow used to aspirate the sample fluid. The limits for the sample draw flow depend on the Syringe volume.

Needle Offset — When a positive value is entered, the needle will not be completely lowered in the sample but will remain above the sample at the distance specified in millimeters.

Upper air gap required — Applies an upper air gap of 1 milliliter.

Lower air gap required — Applies a lower air gap of 1 milliliter.

Calculate button — When the Calculate button is clicked, the upper solvent volume is calculated. The values for Sample volume, Sample draw flow, and the air gaps are copied to the calculated values part of the screen.

Calculations used:

Upper solvent volume = (Loop volume – Sample volume - Upper air gap – Lower air gap) / 2 + Calibrated tubing volume.

Loop volume = ¼ * 3.141593 * (Sample loop inner diameter)^2 * Sample loop length

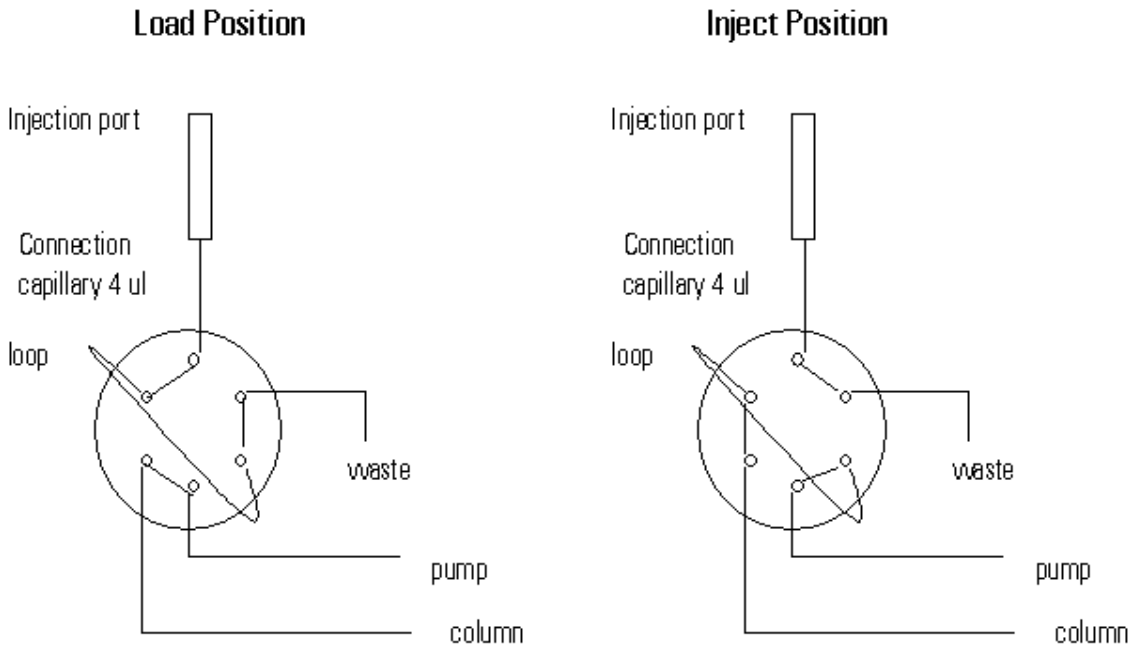
The calibrated tubing connects the injection port to the Rheodyne injection valve. The tubing installed is 4 µl. If you change the size of the tubing, determine the volume and enter this value when using Partial Loop Fill mode.

NOTE Remember to add the capacity of the tubing to the amount of sample when using this task. For example, to draw 10 ml of sample you will require 14 ml (10ml for the sampler loop and 4ml which remains in the tubing).

Partial Loop Fill and Inject

This is a new way of filling the loop and injecting the sample, which does the following:

1 Draws the sample with the specified Sample Volume and Flow.
2 Draws the specified Rinse Volume from the sample.
3 The inject valve is in the Inject position.
4 The Injector is flushed with the Rinse volume.
5 The needle remains in the injector and the valve is switched to Load.
6 The specified sample volume is ejected in the injection port
7 The valve switches to the Inject position and the ChemStation is started.



NOTE The “Partial Loop Fill” task in the previous version of the software is now called “Centered Loop Fill”; see page72.

Rinse Needle Inside / Needle Outside / Injection Port

These tasks should be used either before Draw sample and/or after Inject sample.

Rinse Needle Inside/Outside

The Rinse Needle Inside/Outside task does the following:

1 Moves the needle to the left rinse station.
2 Draws a specified volume from the solvent reservoir.
3 Ejects the volume into the rinse station.
4 Raises the needle to its highest position.

To rinse the inside of the needle, the needle stops above the left rinse station. To rinse the outside of the needle, the needle moves into the deep pocket insert of the rinse station.

Rinse Injection Port

The Rinse Injection Port task does the following:

1 Rotates the injection valve to the inject position.
2 Moves the needle and lowers it into the injection port.
3 Draws a specified volume from the solvent reservoir.
4 Ejects the liquid into the injection port.
5 Raises the needle in its highest position.

Wash Vial

The Wash Vial task does the following:

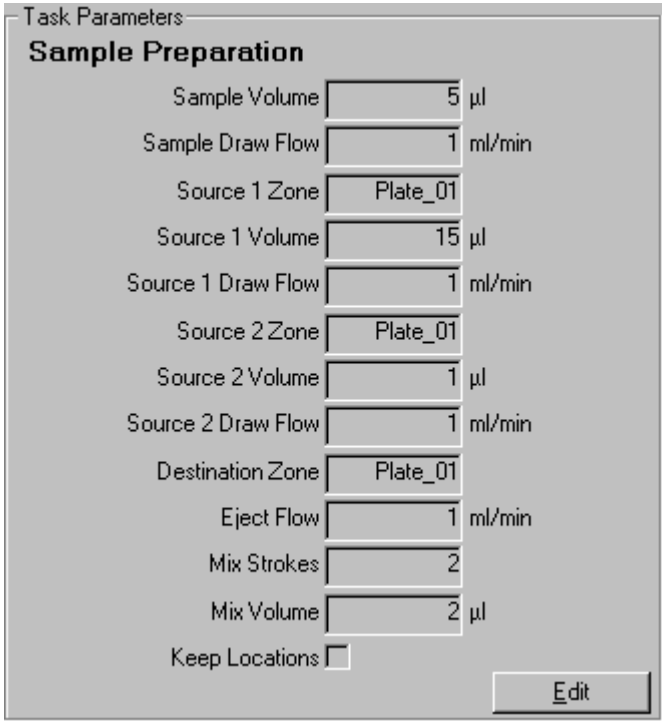
1 Moves the needle to the wash vial located next to the injector.
2 Lowers the needle into the wash vial for the specified time.
3 Raises the needle.

NOTE

Use this task after the Draw Sample task to avoid contamination of the injection port with sample.

Sample Preparation

When using Sample Preparation select preparation plates/racks in the study layout of the Study Parameters screen. This has to be done before you enter the Sampler parameter screen.



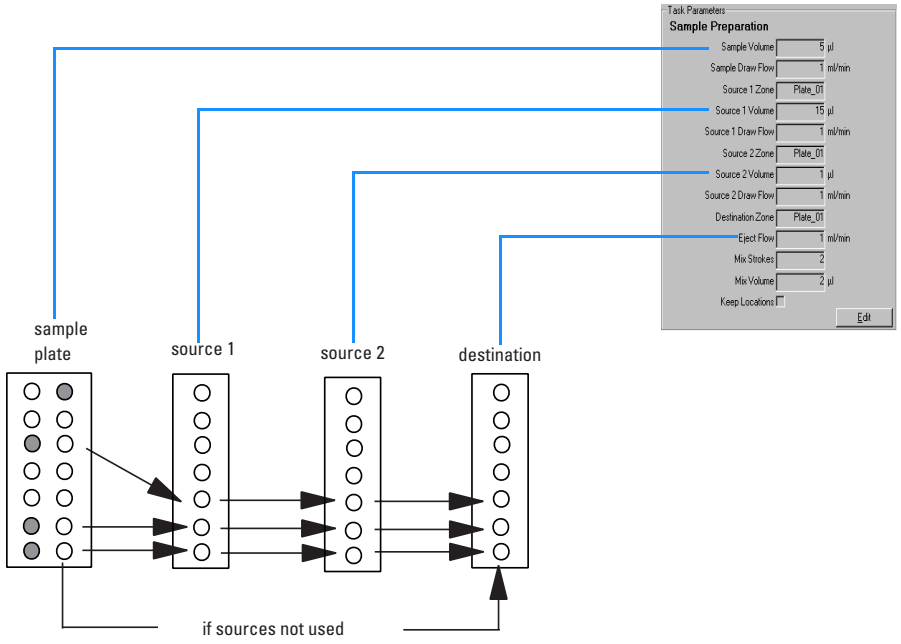
The Sample Preparation task does the following:

1 Moves the needle to the current sample position.
2 Draws the specified Sample Volume with the Sample Draw Flow.
3 Moves the needle to the next position in Source Zone 1.
4 Draws the specified Source 1 Volume with the Source 1 Draw Flow.
5 Moves the needle to the next position in Source Zone 2.
6 Draws the specified Source 2 Volume with the Source 2 Draw Flow.
7 Moves the needle to the first free position in the Destination Zone.
8 Ejects the total volume (Sample Volume, Source 1 and Source 2) into the Destination with the Eject Flow.
9 Draws the specified Mix Volume from the Destination Zone with the Eject flow.

10 Ejects the specified Mix Volume from the Destination Zone with the Eject flow.

11 Repeats steps 9 and 10 as specified by Mix Strokes.

If no other tasks like Draw from Destination and Inject Sample follow the sample preparation task, steps 1-11 will be repeated for all selected samples.



If you select <Not Used> for Source Zone 1 or Source Zone 2, the Zone is skipped.

When the sequence is started, the position in the Source Zones and Destination Zone is the first Well (A:01) or Tube/Vial(1).
Each time the Sample preparation task is called, the next well or tube/vial is used, until all wells or tubes in the rack are used.

Keep Locations

When selected, the next available position is used for source 1 and source 2. The same position as the sample position is used for the destination.

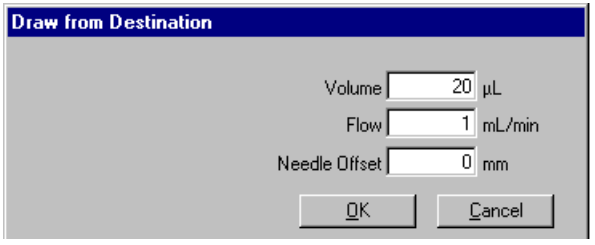
When Keep Locations is *not* selected, the next available position is used for source 1, source 2, and the destination.

Follow these rules for the Sample Preparation task:

- Source 1, Source 2 and Destination must be a Preparation rack or plate. This is defined in the Study Parameter screen
- The number of samples in the study should not exceed the number of wells, available in the preparation rack or plate
- When Keep Locations is selected, the destination rack/plate must have the same layout as the sample plates. Same layout means that the sample plates and the destination plate have equal numbers of wells horizontally and have equal number of wells vertically. They may differ in volume and distance between wells.
- When Keep Locations is *not* selected, the destination rack/plate can be any type

Draw from Destination

This task is used after the Sample Preparation task to draw a specified sample volume from the destination plate. For a sample injection, the Inject Sample task must follow the Draw from Destination task. A needle offset can be selected to enable sampling from a defined vial level.



NOTE The Draw from Destination task can only be used in combination with the Sample Preparation task.

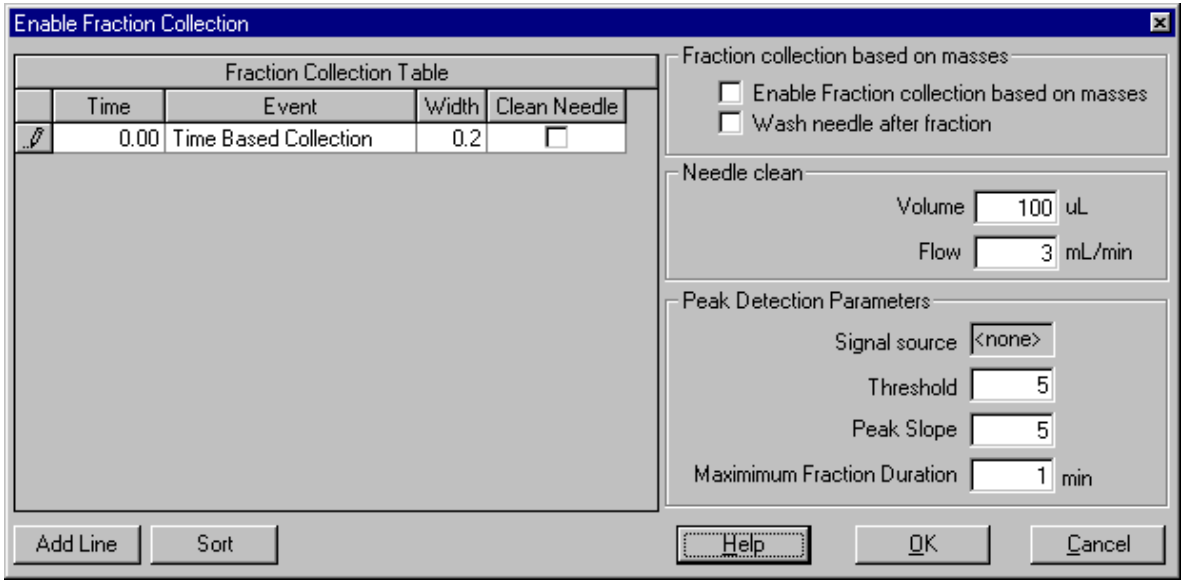
Enable Fraction Collection

CAUTION The Enable Fraction Collection task must be placed before the Inject Sample task.

When using this task, select Fraction Plates/Racks in the Study layout of the Study Parameters screen.

NOTE Be sure that the detector output is set to 0.1 V.

When the system executes the Collect Fraction task, it positions the needle above the next free fraction well of the specified fraction plate or rack. When the criteria for fraction collection are met, the system waits the calculated dead volume time (tube volume / flow) and opens the low pressure valve. The movement of the needle when the well is full depends on options set in the Study parameters dialog box.



Fraction Collection Table

The Fraction Collection table is the main part of the task and consists of four columns. This section is disabled when the Enable Fraction collection based on masses option is selected.

Time The Minutes column holds the times on which the events are taken place.Time 0 is the moment of the injection (when the injection valve is switched to injection). The time is entered in minutes. The maximum resolution is 0.01 minute.

Event If the event which must take place at the specified time. See *Event* below.

Width This entry is only valid for the Time-Based Collection event. For all other events, the text *n/a* appears and the user cannot enter a value. This parameter defines the duration (width) of each slice. The time is entered in minutes. The maximum

resolution is 0.01 minute.

Clean Needle When checked, the needle is cleaned after each fraction. The needle is positioned over the wash station and the inside of the needle is flushed with the volume and flow entered in the Needle clean section. Note that this function is not available for Time-Based Collection mode.

Event

A mix of the following events can be selected.

Peak Detection The fraction collection takes place based on the settings for peak detection. This continues until the next mode or the end of the run occurs. See *Peak Detection Parameters* later in this section.

Start Fraction A fraction is collected until the next mode or the end of the run occurs.

Stop The previous mode is stopped.

Time-based collection Multiple fractions are collected. Each fraction is collected during the time set in the Width column. This continues until the next mode or the end of the run occurs.

The following rules apply to the Fraction Collection table:

1 Duplicated times are not allowed.

2 The last entry in the table must be a Stop event.

3 The Width value only applies to the Time-based Collection event.

4 The Clean Needle flag *cannot* be used with Time-based Collection and Stop events.

Add Line

The Add Line button appends new lines to the Fraction Collection table. The maximum number of in events (Peak Detection, Start Fraction, Time-based collection) in the timetable should not exceed 50.

The Stop event is not counted as an event, this means that you can have up to 50 Start Fraction-Stop events (=100 lines) in the timetable.

Sort

The Sort button sorts the entries in the table in an ascending time order.

Enable Fraction collection based on masses

This function requires the optional Agilent INB 22003 software. Note that the Fraction Collection Table and the Peak Detection Parameters are disabled when this mode is selected.

When the Enable Fraction collection based on masses option is selected, the MSD and the associated parameters in the ChemStation method control the fraction collection. The dead-volume, as specified in the system configuration screen is bypassed. Instead algorithms in the MSD ChemStation automatically determine the dead volume.

Wash needle after fraction

When this option is selected, the needle is cleaned after each fraction collected based on masses. The needle is positioned over the wash station and the inside of the needle is flushed with the volume and flow rate entered in the Needle clean section. This option may interfere with the proper collection of closely spaced peaks.

Needle Clean

Sets the volume and flow rate used to wash the needle after each fraction collected based on masses.

Peak Detection Parameters

Used when the Peak Detection event is selected in the Fraction Collection Table. Peaks can either be collected based on peak detection parameters described below or on mass (see Chapter 6).

NOTE Peak Detection Parameters do not apply to fraction collection based on masses.

The parameters Peak Threshold, Peak Slope and Maximum Fraction Duration are used to detect the peak-start and peak-end for fraction collection. The analog output signal of the detector is continuously monitored by the Control Module, which stores the peak-start and peak-end times. These times are sent to the ChemStation when the run has ended. Each new detected peak is collected in a new well. Depending on the specified microplate type and study parameter settings, multiple wells can be filled from the same peak.

Signal Source The Signal Source box indicate what analog signal the user has selected for peak detection. The selection is made in the System Configuration screen. Possible values are DAD, FLD, VWD, ADC, and <none>. Use <none> when only the MSD is

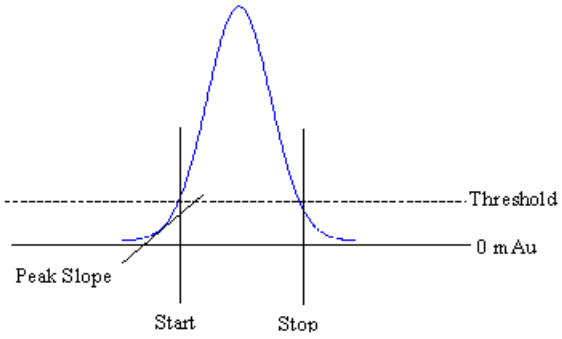
used for fraction collection.

Threshold

The Threshold value is relevant for the peak-start and peak- stop detection. For the peak-start criterion, the peak signal must exceed the threshold before the peak is detected. The peak-stop criterion is met when the peak signal drops below the threshold.

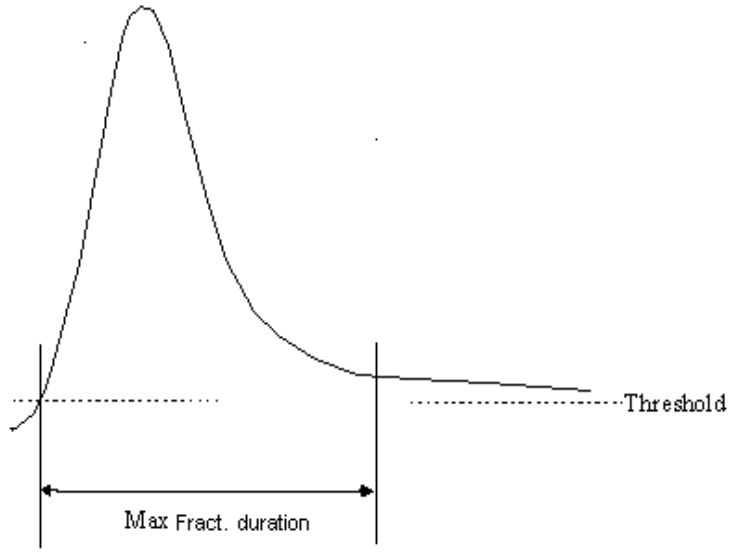
Peak Slope

The slope of the peak signal is calculated using the first derivative out of the last five measured analog values of the peak. The peak-start criterion is met if the peak signal exceeds the threshold AND the slope is equal or greater than than the peak slope setting.



Maximum fraction duration (in minutes)

The Maximum fraction duration parameter can be used to stop the fraction collection in cases when the baseline drifts and the signal does *not* drop below the threshold value.



Enable Fraction Collection Delay Sensor

This task measures the time the fluid needs to travel from the splitter to the sensor:

1 When the run starts, the LP valve is put into the fraction collection position.
2 The needle remains positioned over the rinse station to dispose the fluid.

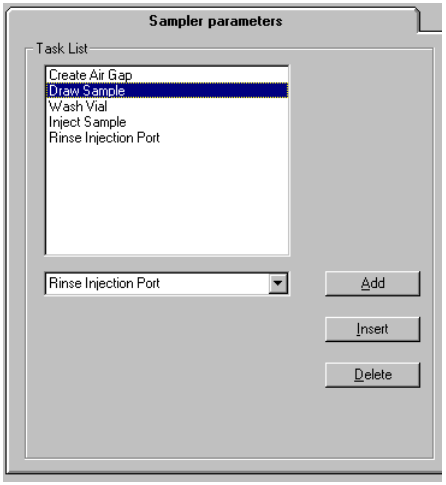
Typically this task is used with the other Sampler tasks listed below.

Draw Sample
Inject Sample
Enable Fraction Coll. Delay Sensor

This task has no parameters.

Examples of sampler task lists

Standard Injection program



This program is recommended when:

- high area precision is required
- enough sample is available to overfill the loop and the calibrated tubing

A sample volume of about 2-3 times of the sample loop installed plus 4µl of the calibrated tubing (the capillary that connects the injection port with the Rheodyne valve) is needed.

Precision with this technique is typically 0.03% to 2% RSD.

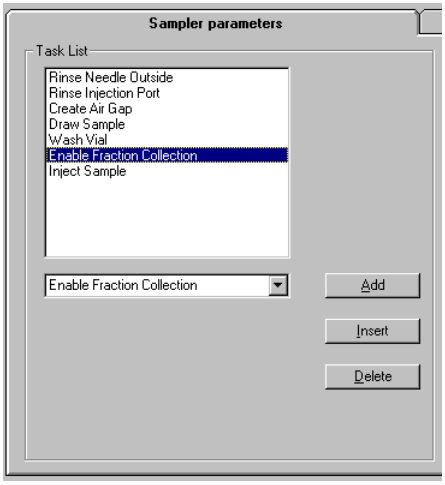
Centered Loop Fill

Task List:

Create Air Gap
Centered Loop Fill
Wash Vial
Inject Sample
Rinse Injection Port

This program is recommended when the sample loop is larger than the desired injection sample. In this case the desired sample volume is introduced into the loop and the remainder volume is filled with solvent. Alternatively the sample can be separated from the solvent by a small air gap.

Sample Injection followed by Fraction Collection



This program is used for sample isolation. Fraction collection is either controlled by peak threshold and slope or by time window.

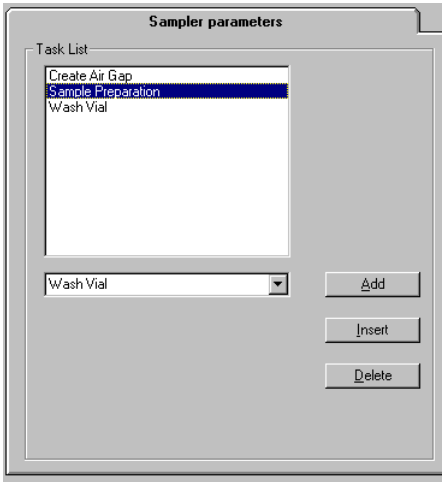
NOTE The Enable Fraction Collection task must be placed before the Inject Sample task.

Select Fraction plates/racks in the Study layout of the Study Parameters screen.

It is recommended that only the same racks or plate type are used for the Fraction

Collection task.

Sample Preparation (without sample injection)



This program can be used for sample preparation like dilution of samples without injection e.g. preparation of daughter plates.

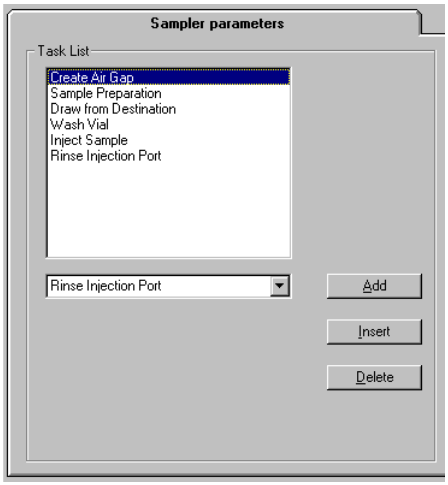
It is strongly recommended that only the same racks or plate type are used for the Sample Preparation task.

CAUTION Only one sample plate or rack for samples are allowed for this task.

Sample Preparation (with sample injection)

This program is used for sample preparation followed by sample injection.

It is strongly recommended that only the same racks or plate type are used for the Sample Preparation task.



CAUTION Only one sample plate or rack for samples are allowed for this task.

To optimize sampler performance

This section describes how to optimize the Agilent 220 Micro Plate Sampler to achieve optimum chromatographic results.

Use the following guidelines to minimize the sample consumption, to eliminate cross-contamination and to maximize injection volume precision, without sacrificing analysis time.

Some of the parameters are entered during the configuration process. The software offers default values according to the type and configuration of your system. Other parameters are submitted with the tasks, as you enter the sampler parameters.

Rinse Needle Inside/Outside, Rinse Injection port, Wash vial

In some instances, sample carry over may become significant when using small injection volumes or injecting a sample of low concentration immediately after a sample of high concentration. The needle rinse tasks move the needle into the rinsing station, wash vial and the injection port between injections, reducing the carry over to a minimum.

The needle wash can be programmed in the Task list. It is strongly recommended that the needle is washed before sample injection in the wash vial which is next to the valve. The main contribution to carry over comes from sample on the outside of the needle. By washing the needle after drawing the sample, the sample is removed from the surface of the needle immediately. When the injection valve switches to Inject position, all of the sample in the sample loop is flushed onto the column. This combination of needle wash and flow-through design reduces carry over to an absolute minimum.

The next needle wash which cleans the needle and the injection port is recommended immediately after sample injection to reduce cycle time.

CAUTION

Don't use any wash steps after sample injection when you want to collect fast eluting peaks. This allows the sampler needle to move directly to the fraction plate after sample injection.

Draw and eject speed

Sample and Solvents

The speed of the draw and eject cycles of the syringe can be adjusted for sample and solvent viscosity. When using viscous samples or solvents, it may be beneficial to reduce the speed of the syringe in order to improve injection volume precision. The speed at which the syringe draws sample out of the well or vial may have an influence on the injection volume precision when using viscous samples. If the draw speed is too high, air bubbles may form in the sample plug, affecting precision. The default draw speed is 1 ml/min. This speed is suitable for the majority of applications, however, when using viscous samples, set the draw speed < 1 ml/min for optimum results.

Air Gap Volume

An air gap is drawn into the needle before the sample is drawn from the well or vial. This ensures that the sample and the liquid in the transfer tubing do not mix. The best compromise for the air gap volume is that it should create an air gap about 10 mm in length inside the transfer tubing. Therefore you should enter: 2 µl for 0.4 mm ID tubing, 3 µl for 0.5 mm ID tubing, 6 µl for 0.8 mm ID tubing, 38 µl for 2.0 mm ID tubing.

Injection Techniques – Loop Filling Modes

The sample loop can be either completely or partially filled. These two techniques differ in accuracy, precision and amount of sample required.

Completely Filling the Sample Loop

This is the conventional method in which an excess of sample is used to fill the sample loop completely. The volume of the loop determines the injection volume. TheAgilent 220 Micro Plate Sampler has the 5 µl loop installed, a 20 µl loop is part of the package. Other loop sizes are available (see Ordering Guide). For this technique use the Draw Sample task.

A 4 µl capillary is connecting the injection port with the Rheodyne valve. This means sample volume must be at least 4 µl plus the sample loop volume.

How Much sample do I Need?

To completely fill the sample loop an excess of sample is required. This is about 2 to 3 loop volumes of sample to achieve 95% of the maximum loop volume (the remainder is residual mobile phase in the sample loop). Determine the optimum number of loop volumes experimentally for your particular application.

Using the 5 µl loop it is recommended to draw at least 20 µl sample (10 – 15 µl plus of the loop volume plus 4 µl of the capillary volume).

Precision with this technique is typically 0.03% to 2% RSD.

Partially Filling the Sample Loop
See also Centered Loop Fill (called Partial Loop Fill in the previous release) on page 72.

Use this method when only small quantities of sample are available.

When you partially fill the sample loop, do not inject more than half of the sample loop volume. As you inject your sample into the loop, the sample mixes with the solvent already in the loop and some sample could be lost if you try to inject too much.

To avoid this you can separate your sample from the solvent by drawing air gaps between sample and solvent.

Precision with this technique is typically 0.02% to 2% RSD.

6

Study Setup for LC/MSD Data Analysis, 92
To set up a ChemStation method to use with CC-Mode, 94
To set up study parameters - LC/MSD, 95
To set up purity calculations, 97
Purity calculation reference, 99
To specify the report format - LC/MSD, 100
To add expected masses to the study list, 102
To calculate the mass from the molecular formula, 104
To import expected masses from a CSV file, 106

Study Setup - LC/MSD Data Analysis

Describes how to set up a study for LC/MSD Data Analysis, including mass-based fraction collection

Study Setup for LC/MSD Data Analysis

This chapter describes how to set up your system to screen for specific compounds, with specified purity levels. See chapter 7 to run a study and chapter 8 to review the data. The following checklist gives an overview of mass-based fraction collection process, as does the figure on the next page.

1 Create a new study.

2 Set up study parameters.

- Select the ChemStation methods.
- If more than one method is to be used, select the conditions under which the next method will be run.
- Edit the purity calculations parameters for the methods.
- Specify the printed report format for the study.

3 Add samples to the study list.

- Click and drag on the displayed plates.

  or

- Import sample information from a CSV file. With the Data Analysis software, expected masses can be imported as numbers or molecular formulas.

4 Specify the expected masses in one of the following ways:

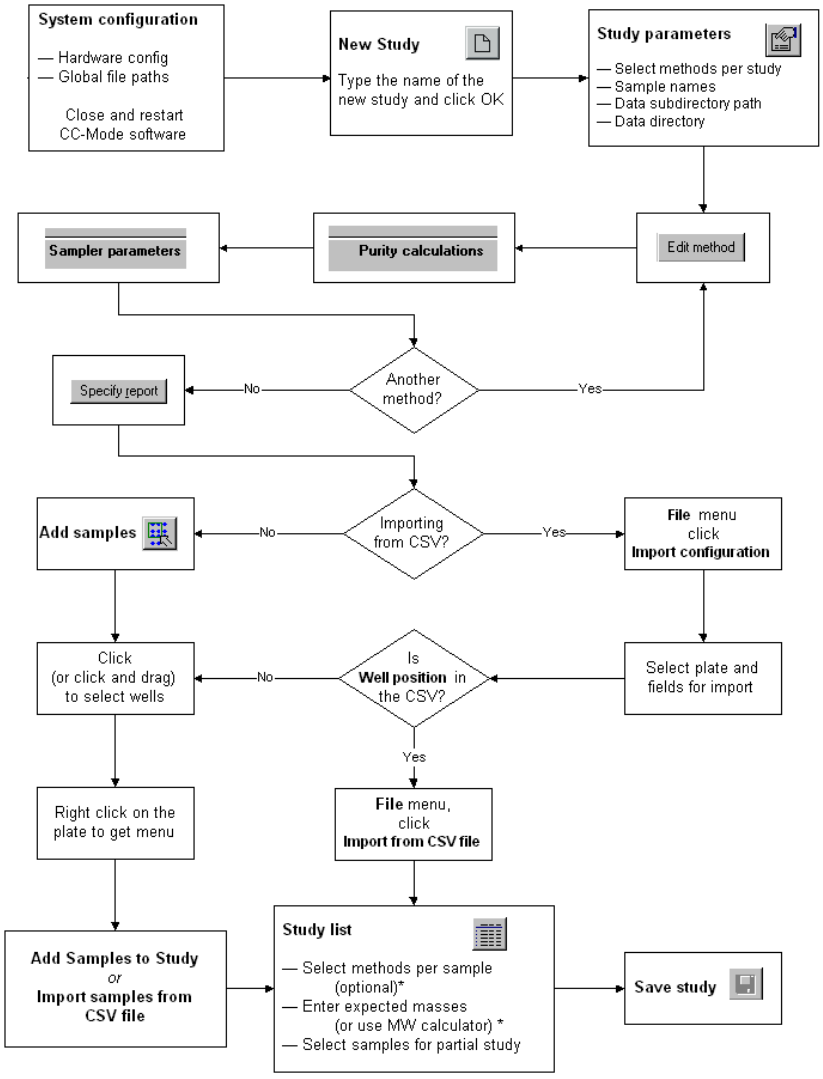
- Type the masses in the **Exp. Masses** tab of the Study list
- Type the chemical formula and use the molecular weight calculator
- Import expected masses or molecular formulas from a CSV file

5 Acquire MSD, MSD and UV, or MSD and “other detector” data.

6 View and report results.

7 (optional) Export results to a CSV file.

Note that some actions will be different if you use the New Study menu item or toolbar button.



* Skip these steps if the information is contained in the imported CSV file.

To set up a ChemStation method to use with CC-Mode

In order to work with CC-Mode, certain conditions must be set correctly in the ChemStation method.

1 **In the Set up MSD Signals dialog box, verify that Use MSD is selected.**

2 **In the Sample Info dialog box, set the Vial to 1 or any other number except a blank or zero.**

The vial number is only used with the 1100 Injector; otherwise the value is ignored.

3 **If you are collecting data with a UV detector or a detector connected via the ADC box in addition to the MS data, enter the Delay time in the Signal Details dialog box.**

4 **If a 35900E ADC is used, verify the proper installation and configuration of the 35900 ADC. Set the configuration of the ADC as follows:**

- 'Sampler type' = other
- 'Vial number' first = 1 last = 1
- 'Send start to sampler' = never
- 'Bottle number' = BCD- 8bit

5 **If you want to generate ChemStation reports for your study, specify a report destination for your method.**

6 **In the Run Time Checklist, verify that Standard Data Analysis is selected.**

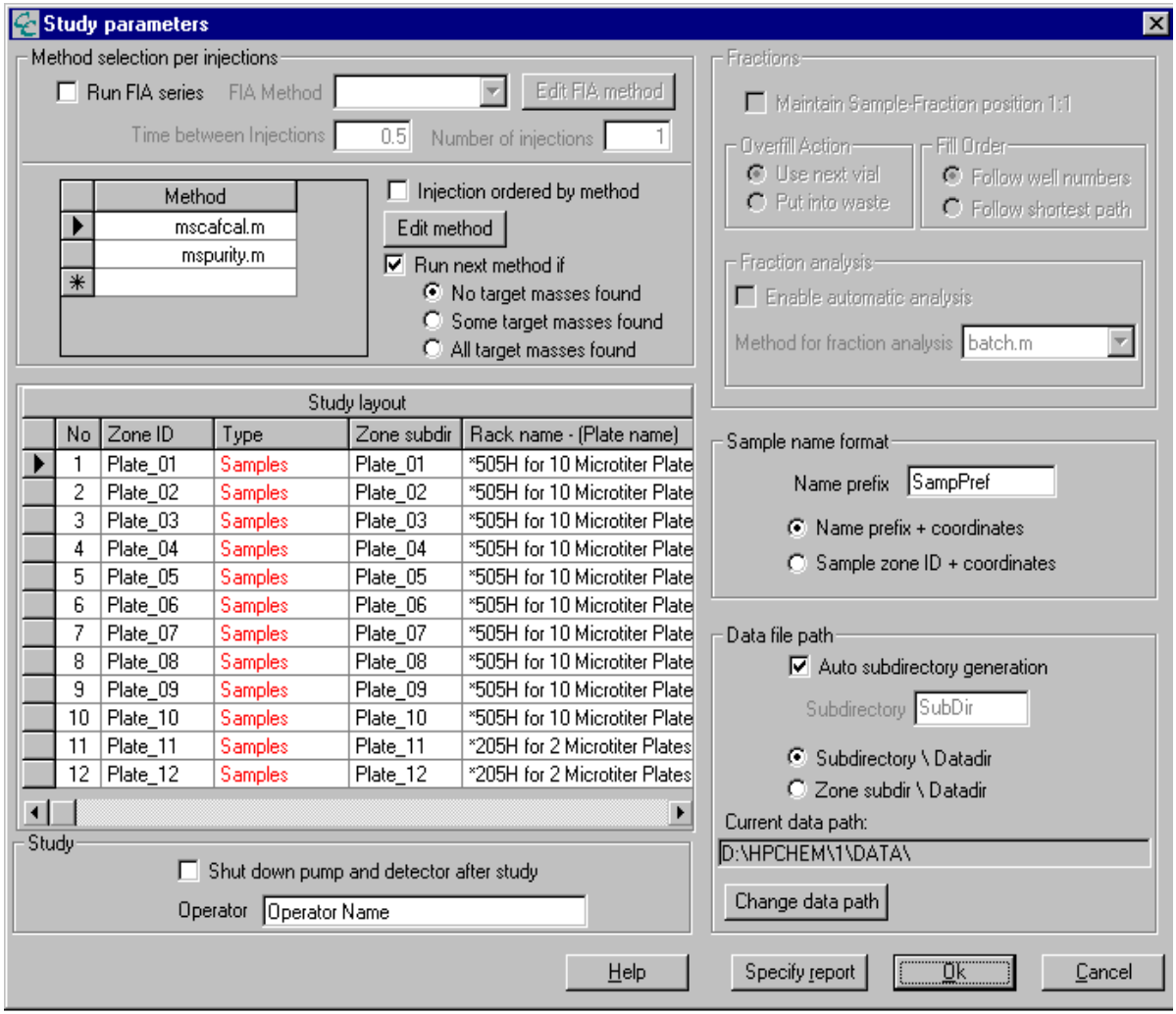
Reprocessing studies changes settings in ChemStation method

If you reprocess an entire study, the following values will be changed in the ChemStation method:

- If you have selected **Disable ChemStation Report** in the Specify Report screen, the report destination will be cleared. You will need to re-select the report destination in order to generate ChemStation reports.

To set up study parameters - LC/MSD

1 **Click the Study parameters icon to display the Study parameters dialog box.**



2 Specify the study parameters as described in *To create a new study* on page 42.

3 Specify the LC/MSD analysis study parameters described below.

Injection ordered by method

When this option *is* selected, all methods specified for each sample are run unconditionally. The conditions set for running the next method described below are ignored.

Run next method if

If you want all the methods to be run, leave the **Run next method if** box blank. Otherwise, select the **Run next method if** option and choose one of the following conditions from the data of the first method for running the next method,

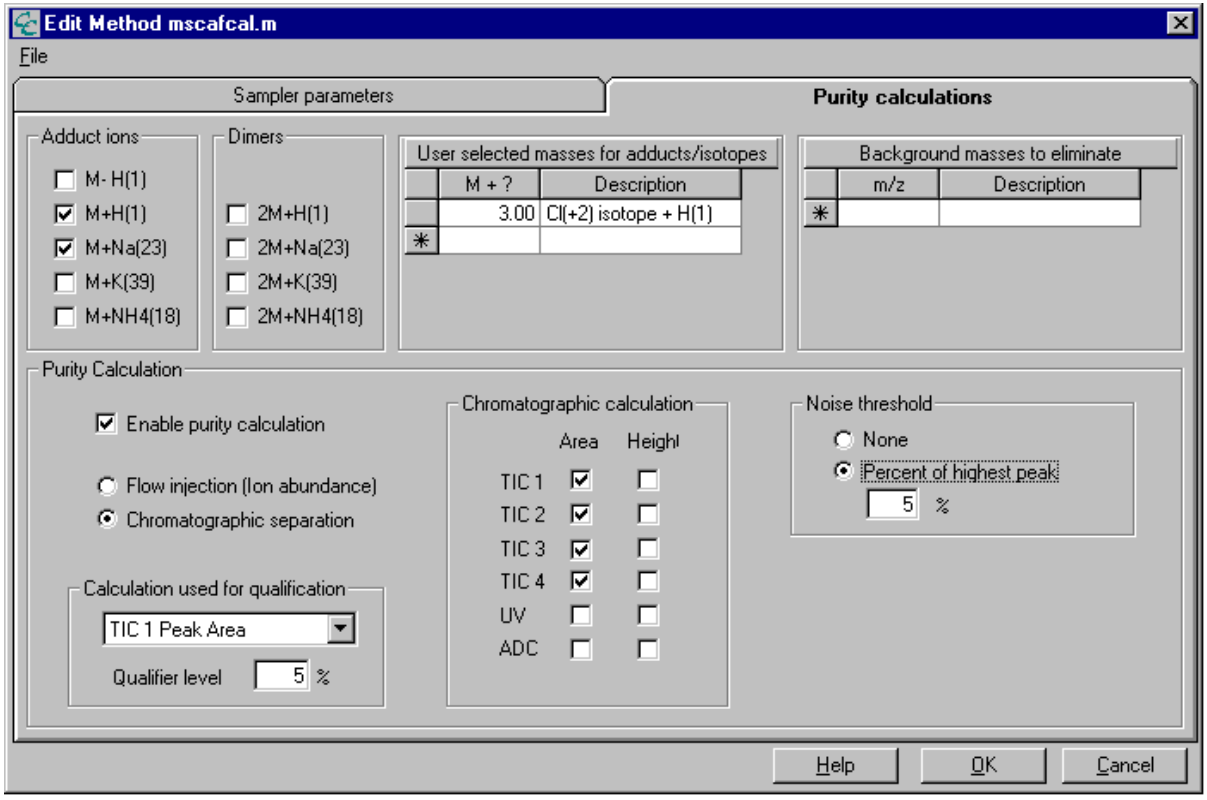
- No target masses found
- Some target masses found (at least one)
- All target masses found

4 Proceed to *To set up purity calculations*, 97.

To set up purity calculations

1 **In the Study parameters screen, click Edit Method.**

2 **Click the Purity calculations tab on the Edit Method dialog box.**



3 **Select the expected adduct ions and dimers.**

If you are not sure which adducts and dimers might be formed from your compound, select any adduct that might occur under your analytical conditions.

4 **In the User selected masses for adducts / isotopes field, enter any other expected ions.**

Click on the cell next to the asterisk character (*) and enter the value for mass.

5 Enter any background masses to subtract from the calculations.

To determine background ions, run a blank and examine the mass spectra.

6 Select Enable purity calculation.

Otherwise, no purity calculations will be performed.

7 Check the appropriate analytical technique and associated parameters.

Flow injection (ion abundance)

The individual components in the sample are not separated. The purity calculation is done by using the ion abundances of an averaged mass spectrum. This type of calculation is only appropriate for mass spectral data. The formula used to calculate the purity is described on page 99.

Chromatographic separation

Normal chromatographic separation used in the analysis. Specify Chromatographic calculations, the Calculation used for qualification, and the Qualifier level (percent purity) as described below. The formula used to calculate the purity is described on page 99.

Chromatographic calculation

Select which calculations will be performed on chromatographic data. Note that signals from UV or other detectors may be used here, in addition to MS data.

Calculation used for qualification

Specify which chromatographic calculation to usefor qualification. For FIA, **Ion abundance** is the only option; for chromatography, any of the options selected under **Chromatographic calculation** can be used.

Qualifier level

Enter the percent purity that the selected signal must exceed in order to be considered present in the sample (“found”). Adjust the Qualifier level to reflect the number of compounds and the relative amount of each compound expected. For example, for an equimolar mixture of four compounds, the highest qualifier level possible for any of the four compounds would be 25% if you had 100% yield for each; since 100% yield is unlikely in most cases, set the Qualifier level around 15%.

8 Enter the noise threshold.

In FIA, this parameter is calculated as the percentage of the most abundant ion. For chromatography, the noise threshold is calculated as the percentage of the signal selected for the qualifier.

9 Click OK to return to the Study parameters screen.

Purity calculation reference

All signals below the noise threshold are ignored. That is, only signals above the noise threshold are used in the calculations.

FIA purity calculation

Taking the averaged spectrum across the largest peak in the TIC at half-height, the purity is calculated by summing the abundances of all the selected ions and dividing this sum by the sum of all the ions. This ratio is multiplied by 100 for percent purity.

$$\% \text{ purity} = \frac{\sum \text{selected ions}}{(\sum \text{all ions}) - (\sum \text{all background ions})} \times 100$$

Chromatographic separation purity calculation

All chromatographic peaks are first integrated. The purity calculation depends on whether **peak area** or **peak height** was selected.

For mass spectral data, an extracted ion chromatogram (EIC) for each selected ion is generated. The sum of the integrated peak height or area in the EIC for each selected ion is divided by the sum of the TIC (total ion chromatogram). This ratio is multiplied by 100 for percent purity.

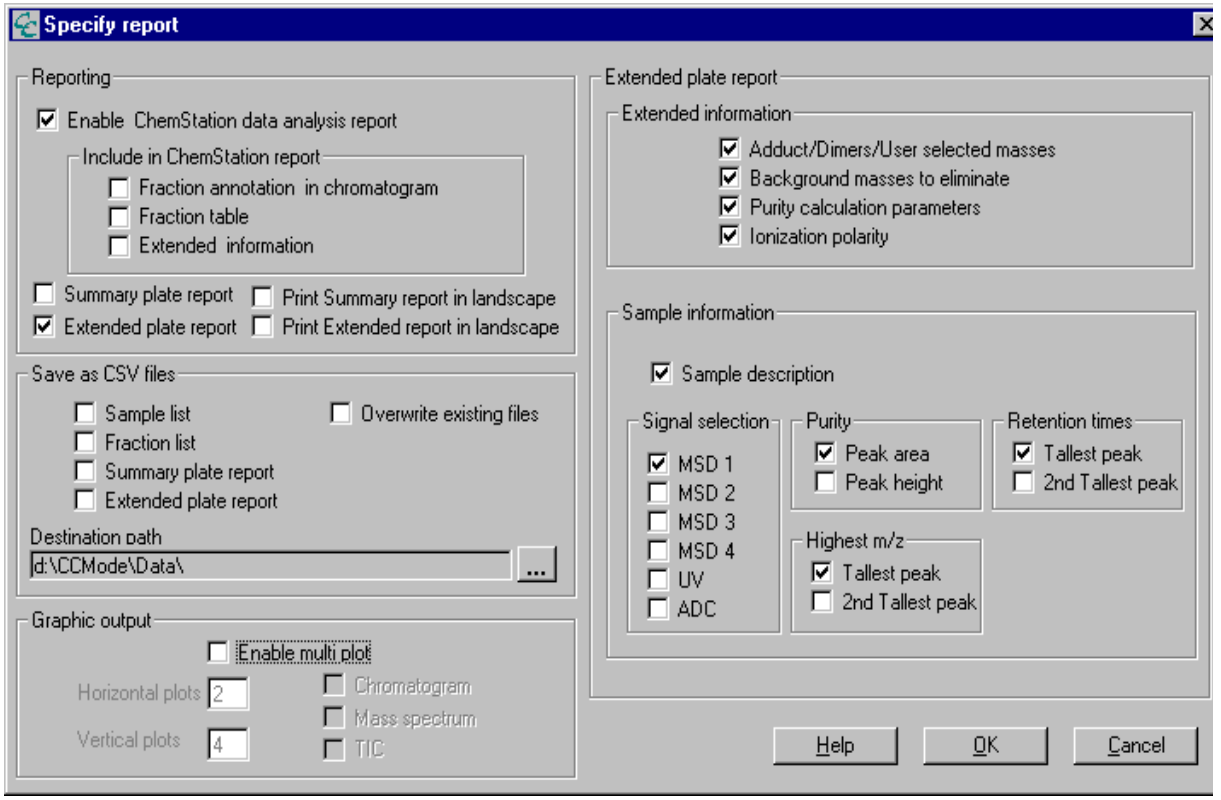
$$\% \text{ purity} = \frac{\sum \text{peaks in EIC}}{(\sum \text{all peaks in TIC}) - (\sum \text{background peaks})} \times 100$$

For UV or ADC data, the software uses an EIC for each target mass to locate each peak of interest. The area or height of the peak of interest is divided by the sum of the peaks in the chromatogram. This ratio is multiplied by 100 for percent purity.

$$\% \text{ purity} = \frac{\text{peak of interest}}{\sum \text{all peaks in chromatogram}} \times 100$$

To specify the report format - LC/MSD

1 In the Study parameters screen, click Specify report.



2 Select the Enable ChemStation Data Analysis report option if you want to print a complete ChemStation report for every sample.

The report will be generated using the report style specified in the ChemStation method. If not selected, ChemStation report are *not* generated.

3 Select any optional CC-Mode information you want printed with the ChemStation report (if applicable.)

4 Select either Summary plate report or Extended plate report.

5 If you selected Extended plate format, select the items in the Extended Plate Information section to include in the report.

Note that "Highest *m/z*" refers to the ion with the largest *m/z* value, above the threshold, usually the molecular ion. This should *not* be confused with the ion with the largest abundance (base peak).

6 If you want a graphical report printed, select Enable multi-plot.

This will cause the selected graphics to be printed in the chosen arrangement. Results from successive samples are plotted continuously, without a page break between samples. The number of plots per page is determined by theHorizontal plots and Vertical plots parameters. For example, if Horizontal plots is set to 3 and Vertical plots is set to 4, 12 plots are printed per page. The number of plots per sample depends on which options have been selected. In the case of chromatographic data with more than one expected mass, you will get one plot for each mass.

Chromatogram When selected, the UV chromatograms are printed on the report.

Mass spectrum When selected, the mass spectrum for each target mass is placed on the multi plot page. Depending on the type of analysis this is either:

- Chromatographic data - Spectrum at half-height of the largest EIC peak for each target mass.
- FIA data - Averaged spectrum of the single peak at half-height.

TIC When selected, the TIC (total ion chromatogram) is placed on the multi plot page.

The availability of these options depends on which software products are installed.

7 Select the types of data for which you want to generate graphical reports.

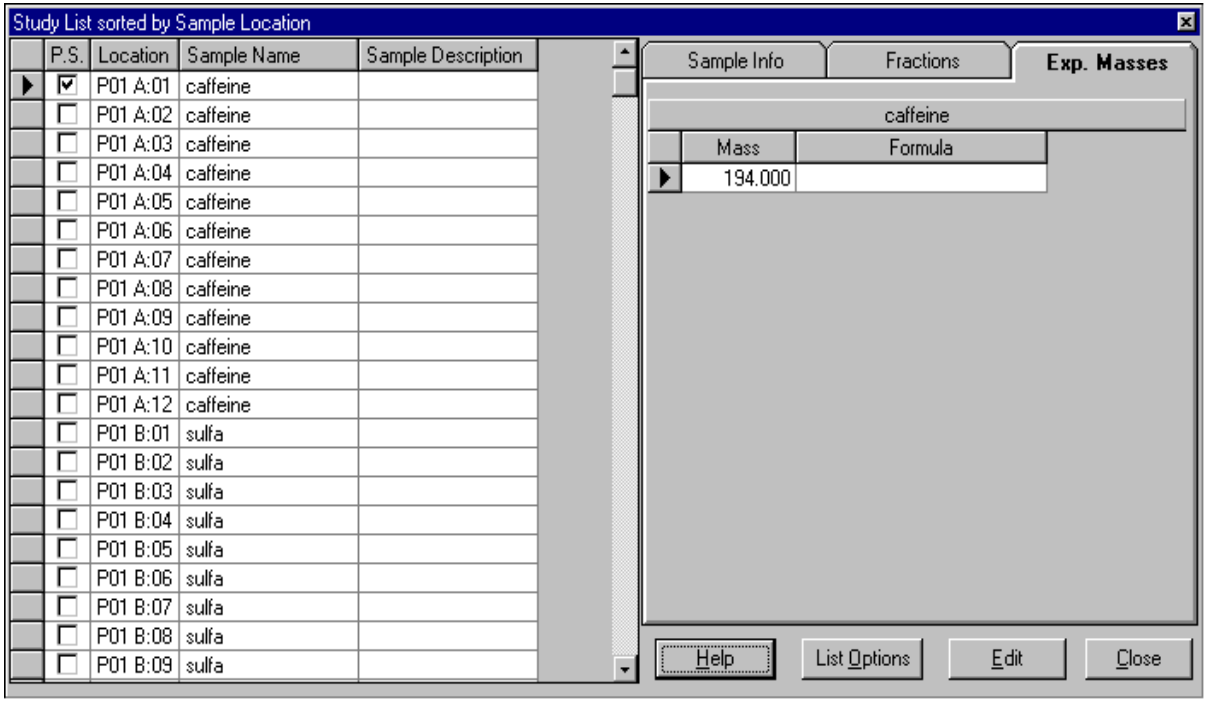
- UV chromatogram
- Mass spectrum
- TIC (Total Ion Chromatogram)

8 Click OK to close the dialog box after setting all desired report options.

To add expected masses to the study list

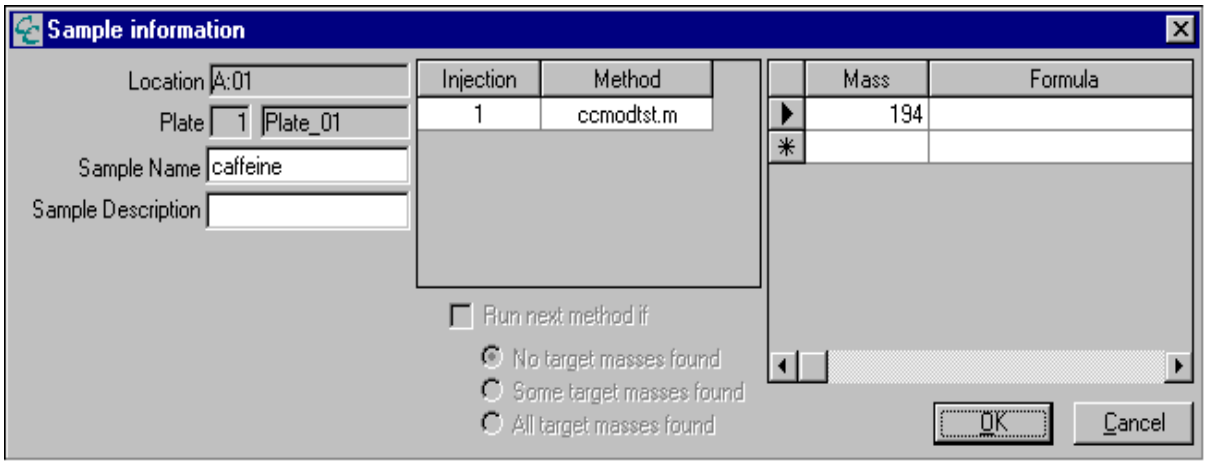
1 **Click the Study List toolbar button.**

2 **Click the Exp. Masses tab.**



3 **Click the Edit button.**

The following dialog box appears.



4 Enter the monoisotopic molecular weight for each expected compound in the Mass column, then click OK.

5 Add new lines to the Mass table by clicking on the cell next to the * character and entering in the new mass.

See also ***To calculate the mass from the molecular formula on page 104***.

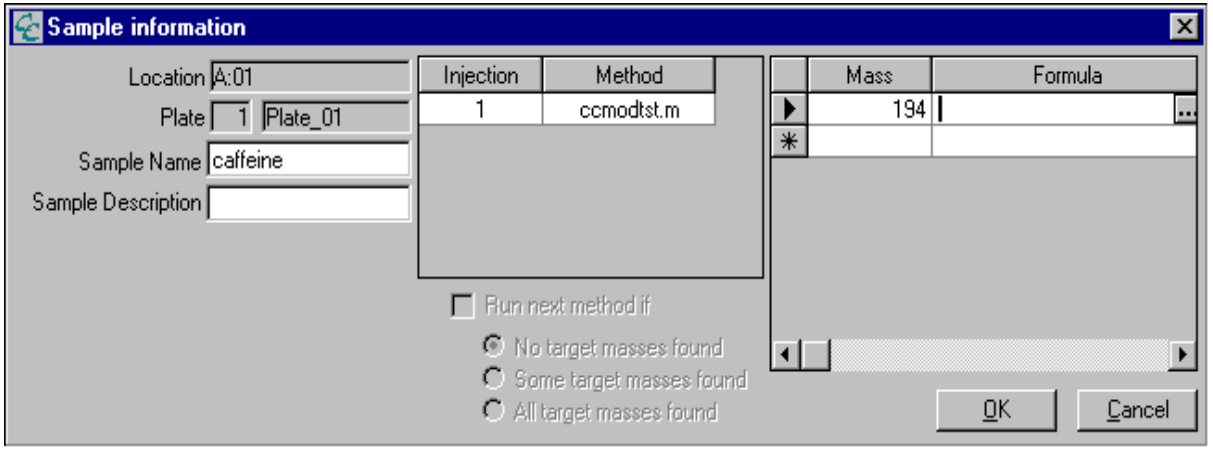
To calculate the mass from the molecular formula

1 **Click on the Study List button.**

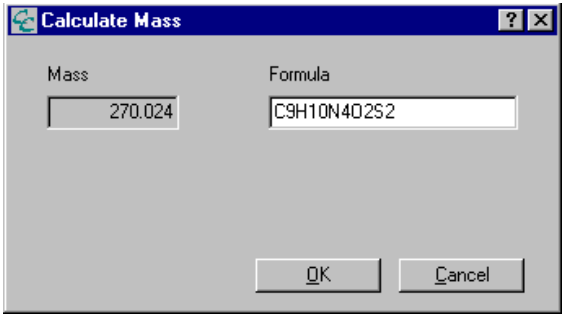
2 **Click the Exp. Masses tab.**

3 **Click the Edit button.**

4 **Click in the Formula field. An ellipsis** **button appears on the line you clicked as shown in the example below.**



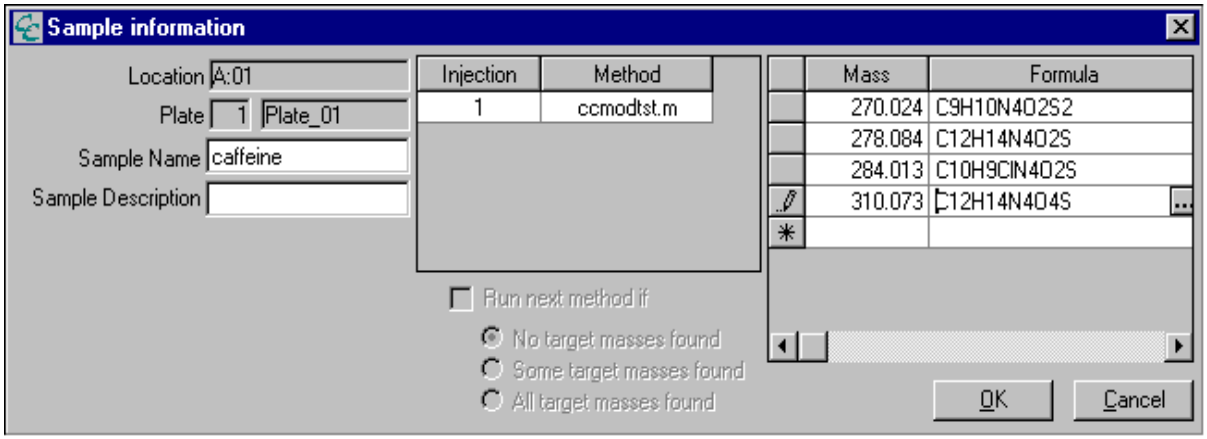
5 **Click the ellipsis** **button to display the Calculate Mass dialog box shown below.**



6 Type in the formula field, then click OK.

The molecular weight calculator is case-sensitive; use upper and lower case correctly.

7 Repeat steps 4 through 6 for each formula.



8 Click OK to close the Sample Information dialog box.

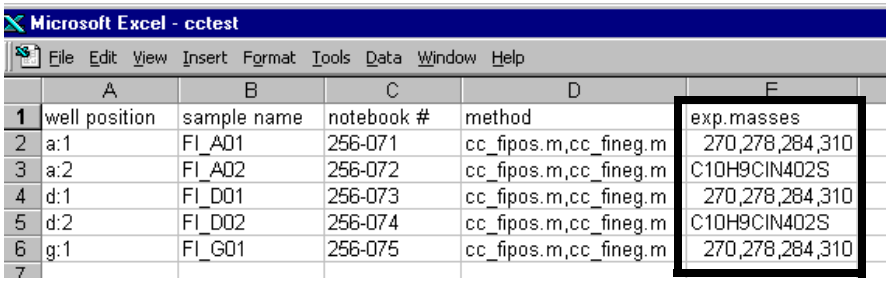
9 Click Close to close the Study List dialog box.

To import expected masses from a CSV file

Expected masses can be imported either as monoisotopic molecular weights or as molecular formulas. The software will automatically calculate masses from the formulas.

1 **If you are using Excel, set up your spreadsheet with all the expected masses for each sample in the same cell, separated by commas.**

Molecular formulas must be entered using correct capitalization.



	A	B	C	D	E
1	well position	sample name	notebook #	method	exp.masses
2	a:1	Fl_A01	256-071	cc_fipos.m,cc_fineg.m	270,278,284,310
3	a:2	Fl_A02	256-072	cc_fipos.m,cc_fineg.m	C10H9ClN402S
4	d:1	Fl_D01	256-073	cc_fipos.m,cc_fineg.m	270,278,284,310
5	d:2	Fl_D02	256-074	cc_fipos.m,cc_fineg.m	C10H9ClN402S
6	g:1	Fl_G01	256-075	cc_fipos.m,cc_fineg.m	270,278,284,310
7					

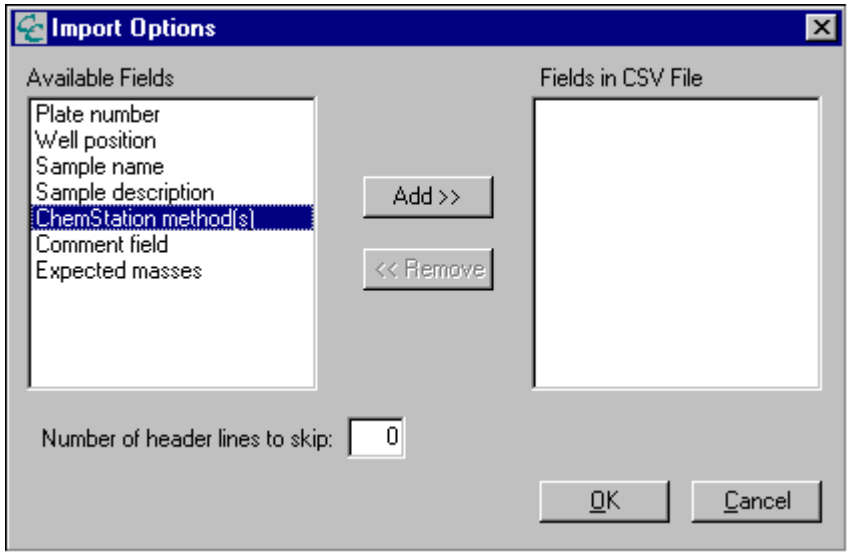
2 **Export your Excel spreadsheet in .csv file format.**

Export to the path specified in the system configuration for easiest use.

The CSV file from the spreadsheet above looks like this if you view it in Notepad. Note that multiple values per cell (and per field) are grouped within quotation marks.

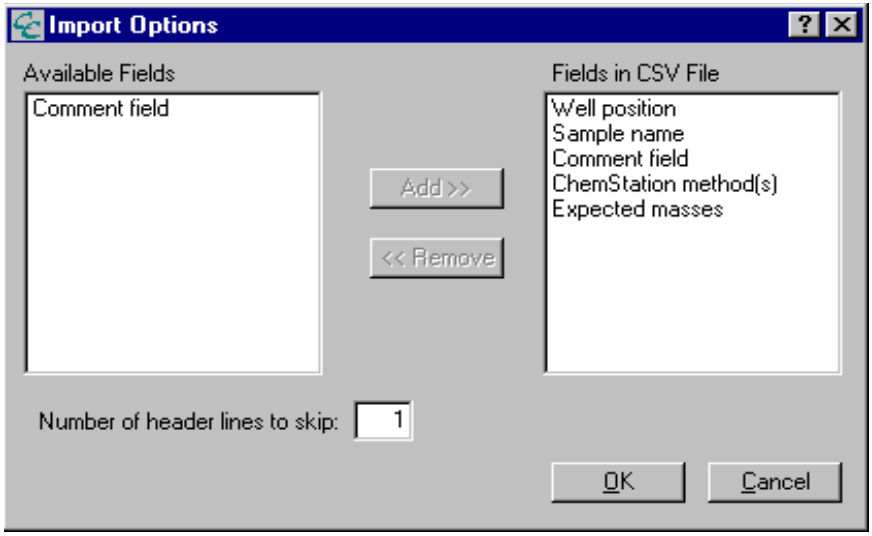
```
well position,sample name,notebook #,method,exp.masses
a:1,Fl_A01,256-071,"cc_fipos.m,cc_fineg.m","270,278,284,310"
a:2,Fl_A02,256-072,"cc_fipos.m,cc_fineg.m",C10H9ClN402S
d:1,Fl_D01,256-073,"cc_fipos.m,cc_fineg.m","270,278,284,310"
d:2,Fl_D02,256-074,"cc_fipos.m,cc_fineg.m",C10H9ClN402S
g:1,Fl_G01,256-075,"cc_fipos.m,cc_fineg.m","270,278,284,310"
```

3 **Select Configure CSV file layout from the File menu in the CC-Mode Main window.**



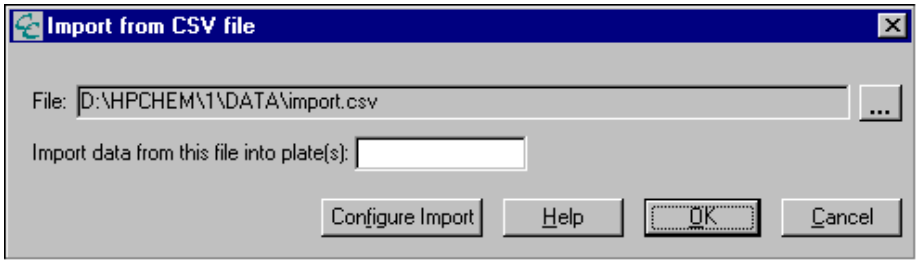
4 **Move the names of the fields you wish to import from Available Fields to Fields in CSV File in the order in which they occur in your CSV file, as shown in the example below.**

Use **Comment field** to identify any fields that should not be imported.



5 **Click OK to exit.**

6 **On the File menu, click Import from CSV File.**



7 **If your CSV file was not saved in the default path, use the ... button to locate it.**

8 **Enter the number of the plate for this file.**

9 **Click OK to exit.**

To set up for fraction collection - LC/MSD

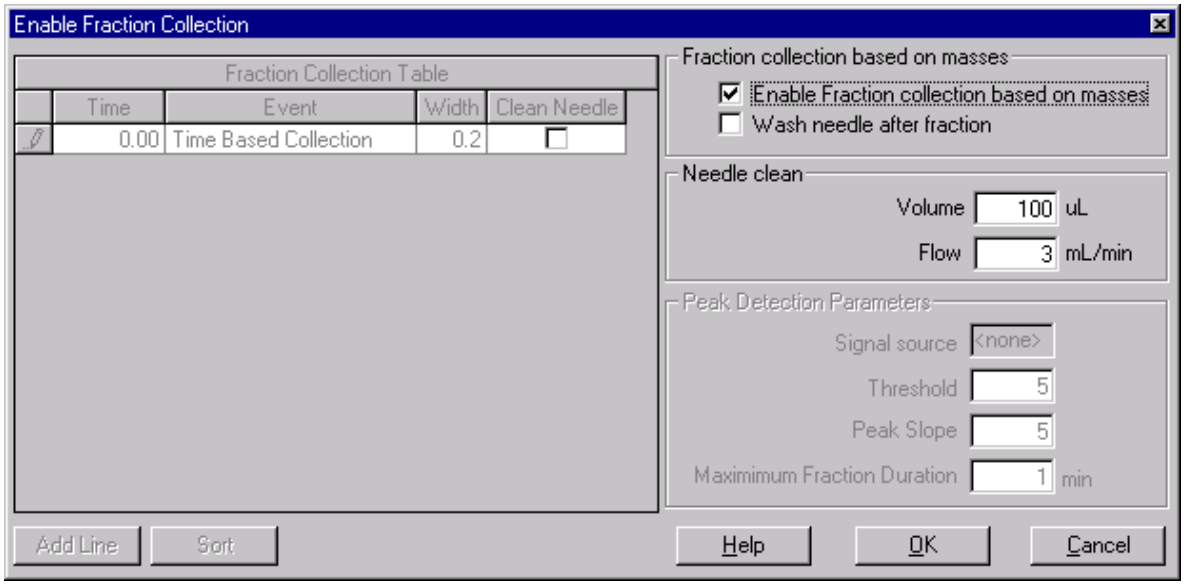
Mass-based fraction collection requires Agilent INB 22003 software.

1 **Select the Edit Method button from the toolbar in the CC-Mode Main window (or click the Edit Method button from the Study Parameters dialog box).**

2 **Select Enable Fraction Collection from the Sampler Parameters task list, then click Add.**

3 **Click the Edit button to display the Enable Fraction Collection dialog box shown below.**

4 **Select the Enable Fraction collection based on masses option as shown in the example below.**



When the Enable Fraction collection based on masses option is selected, the MSD and the associated parameters in the ChemStation method control the fraction collection. The dead-volume set in the System Configuration screen is bypassed. Instead the MSD ChemStation software automatically determines the dead volume.

The timetable and Peak Detection parameters are disabled when this mode is selected.

5 (optional) Select the Wash needle after fraction option.

When this option is selected, the needle is cleaned after each fraction collected based on masses. The needle is positioned over the wash station and the inside of the needle is flushed with the volume and flow entered in the Needle clean section.

CAUTION

The Wash needle after fraction option may interfere with proper fraction collection, depending on how your method parameters are set. *Don't* use this option if the method is set up to collect fractions close together.

6 Click OK to close the Enable Fraction Collection dialog box.

See Also

The fraction options on the Study parameters screen are described in *To set up for fraction collection* on page 61.

7

To set up a ChemStation method to use with CC-Mode, 112
To set up a ChemStation FIA method to use with CC-Mode, 114
To run a study, 115
To monitor the status of a run, 117
To analyze fractions, 119
To automatically analyze fractions, 120
To pause or stop a study, 121

Data Acquisition and Fraction Collection

Describes how to run and monitor studies, fractions and FIA analyses

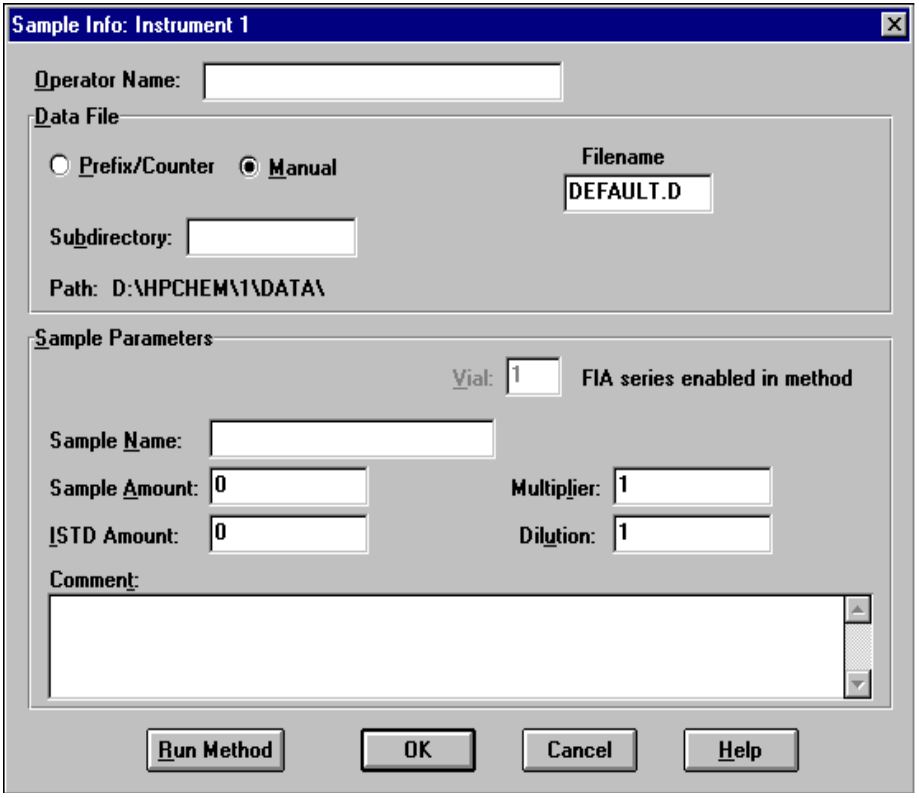
To set up a ChemStation method to use with CC-Mode

In order to work with CC-Mode, the following parameters must be set correctly in the ChemStation method.

1 **Set up your 1100 Series LC modules for analysis.**

- Set the Data File to Manual
- Set the Vial to 1 or any number except a blank or zero

These parameters can be set on the Sample Info dialog box, which is accessible from the RunControl menu in the Method & Run Control view of the ChemStation.

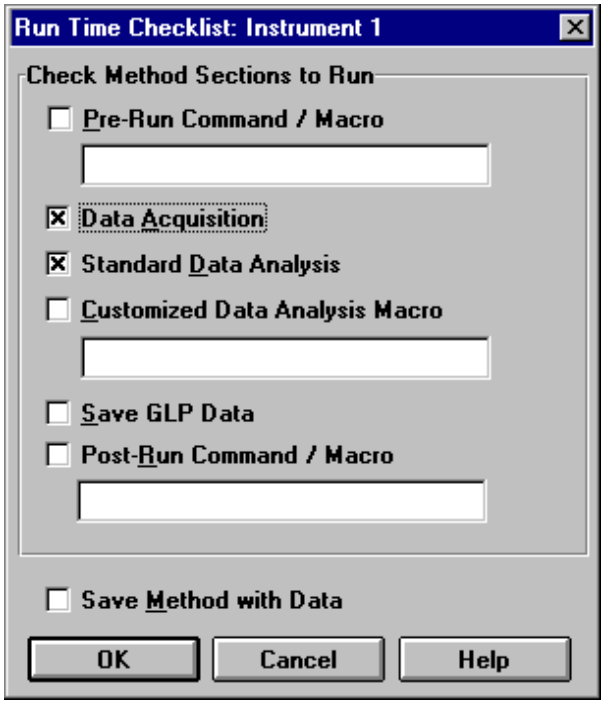


2 **If you want to generate ChemStation reports for your study, specify a report destination for your method.**

You can set the report destination field on the Specify Report dialog box, which is accessible from the Report menu in the Data Analysis view.

3 In the Run Time Checklist, verify that Standard Data Analysis is selected.

You can access the Run Time Checklist dialog box from the Method menu in the Data Analysis view.



Reprocessing

CAUTION Reprocessing studies changes settings in ChemStation methods.

If you reprocess a whole or partial study, the following value will be changed in the ChemStation method:

- If you have selected Disable ChemStation Report in the Specify Report screen, the report destination will be cleared. You will need to reselect the report destination in order to generate ChemStation reports.

To set up a ChemStation FIA method to use with CC-Mode

In addition to the items mentioned in the previous topic, *To set up a ChemStation method to use with CC-Mode*, do the following steps for FIA.

1 **With the method of interest loaded, select Edit FIA Series from the Method menu in the Method & Run Control view.**

The Edit FIA Series dialog box appears.

2 **Select Enable FIA Series in Method option on the Edit FIA Series dialog box.**

3 **Save the ChemStation method.**

Make sure the method runs isocratic mobile phase conditions, rather than gradient, for FIA.

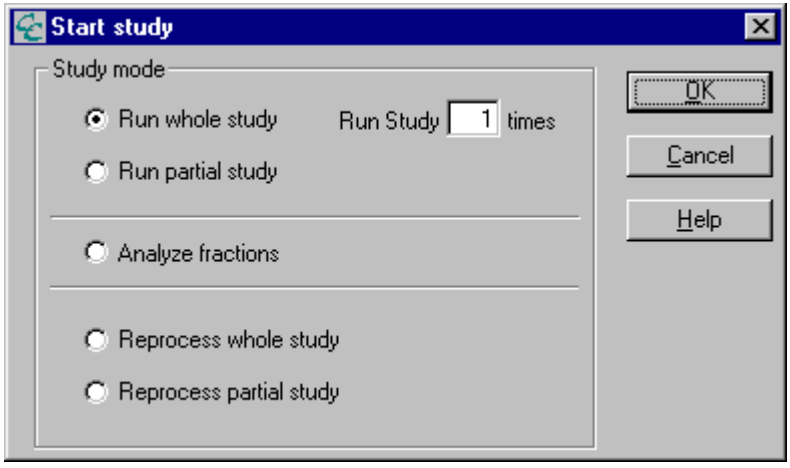
To run a study

The name of the current study is displayed in the title bar of the CC-Mode Main window. To run the current study, skip to step 3.

1 To open a different study, select Open Study from the File menu.

2 Click on the name of the study you want to run in the list, then click OK.

3 **Click the Start Study button on the toolbar of the CC-Mode Main window to display the following dialog box.**



4 Select one of the following options to run a study:

- Run whole study
- Run partial study

Pooling

Use the Run Study n times parameter if you want to collect the same fraction multiple times from the same sample into the same vial. This has the effect of purifying the component in the fraction. This is most commonly done to get enrichment of a trace component but can also be done for a major component. When using this technique, also select the Maintain Sample Fraction Position 1:1 option on the Study parameters dialog box during study setup. This option causes the system to collect the fractions in the corresponding well position as the sample. Note that in

this case, the shape of sample and fraction plates must be the same (same number of rows and columns). Also, there must be a fraction plate for every sample plate.

5 Click OK to start the study.

The samples will be analyzed and any reports you specified will be generated.

FIA study

For FIA, a single ChemStation report will be generated at the end of the study, rather than after each injection.

Note

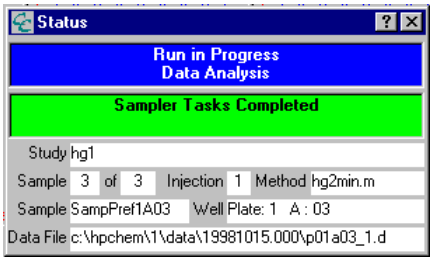
Don't use the ChemStation Run Method or Run Sequence options while a CC-Mode study is running.

To monitor the status of a run

The current status of the system can be viewed from the status screen and the status bar, which is at the bottom of the CC-Mode main window.

Status screen

➤ To open the Status screen, click the red icon in the menu bar.



➤ Access the Sample Info screen in the ChemStation software and verify that the Data File is set to Manual and that the Vial is set to 1.

The Status screen displays information about what is happening with the ChemStation and the 220 Sampler while the system is running. This includes the name of the sample being analyzed, the Study name, the plate/rack name, the well position and the corresponding data file name.

The Status screen can be placed anywhere on the screen by clicking on the blue title bar and dragging the window to the desired position, while holding down the left mouse button. To remove the Status screen click on the cross in the top right corner.

Status bar

➤ Use the status bar at the bottom of the main window to monitor the progress of a run.

The following information is displayed (from left to right) in the status bar:

- Mode: View mode/select mode
- Location of the current sample well
- Sample name of the current sample well
- The data file name of the current sample
- CC-Mode status
- Sampler status

Fraction

When a fraction is being run, the status bar shows the fraction origin and the sample origin, name and data file of the sample that the fraction was collected from.

To analyze fractions

1 **In the Study Parameters screen, select the ChemStation method for analyzing your desired fractions. Or select the ChemStation method after selection of fractions in the Study list, then click OK.**

NOTE

It is not possible to analyze fractions with activated Enable Fraction Collection task in the Sampler Parameters.

2 **In the Main screen, click with the right mouse button on the (green) fraction well of interest.**

3 **Repeat this for each fraction you want to inject.**

4 **Right-click on one of the fraction plates. Select Mark Fractions for Analyze from the pop-up menu.**

The fractions that you add to the study turn blue on the screen.

5 **Click the Start Study button on the toolbar of the CC-Mode Main window to display Start Study dialog box.**

6 **Select Analyze fractions, then click OK.**

The fractions will be analyzed and any reports you specified will be printed.

To automatically analyze fractions

Use the following procedure to set up the system to automatically analyze fractions at the end of the run.

1 **Select the Enable automatic analysis option on the Study parameters screen.**

All collected fractions will be automatically injected when a study ends.

2 **Select the method for fraction analysis.**

The selected method will be used to analyze the fractions that were collected during the study.

NOTE The selected method should not have Enable Fraction Collection in the Sampler task list.

To pause or stop a study

You can pause or stop a study that is currently running as follows:

To pause a study

Click the pause button. You will see a message box to confirm. When the acquisition for the current method ends, CC-Mode enters the Pause State.

To resume a study

A study that has been paused as described above can be restarted by clicking the Resume toolbar button shown below.

To stop a study

Click the Stop button while the study is paused as described above. This ends the study.

To pause or stop a study

8

To review the results of a study, 124
To view the results of a study for individual samples, 125
To view the sample/fraction relation, 129
To review FIA results, 130
To reprocess a study, 132
To print or export results, 133
To review CC-Mode results remotely, 135

Data Review

Describes how to review analytical results and print or export them

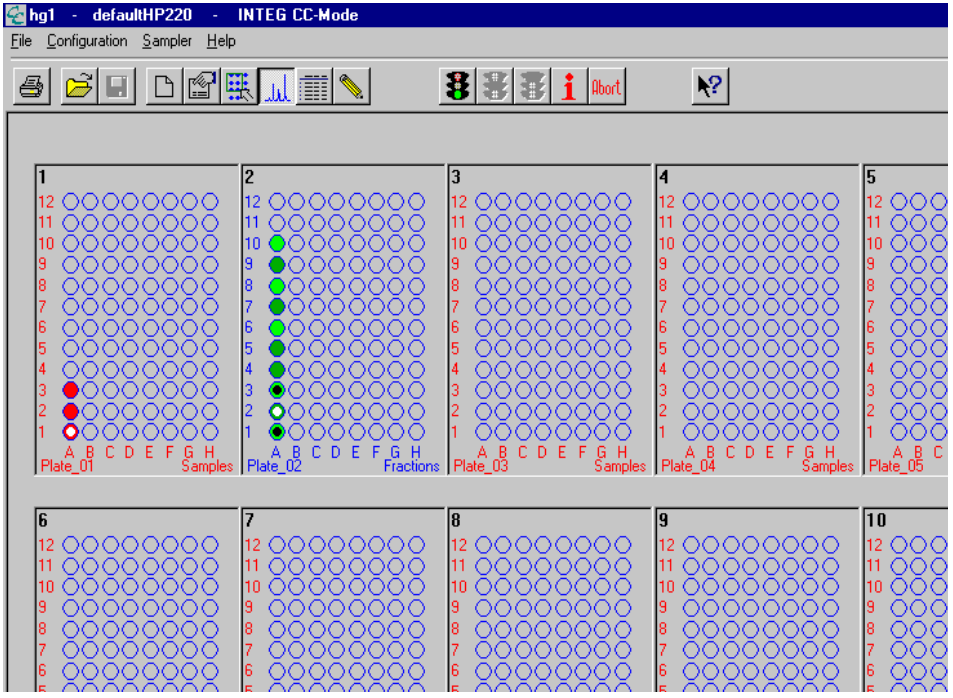
To review the results of a study

The main window of CC-Mode shows a diagram of all configured plates and racks. After a study has been run, all sample wells and fraction wells will be color-coded.

- Red = sample wells
- Green = fraction wells (alternate light and dark green. Adjacent wells that belong to the same peak will have the same color, then the color will change for the next peak).
- Blue = fraction wells marked for reanalysis (alternate light and dark blue. Adjacent wells that belong to the same peak will have the same color, then the color will change for the next peak).

For LC/MSD Data Analysis (INB 22002):

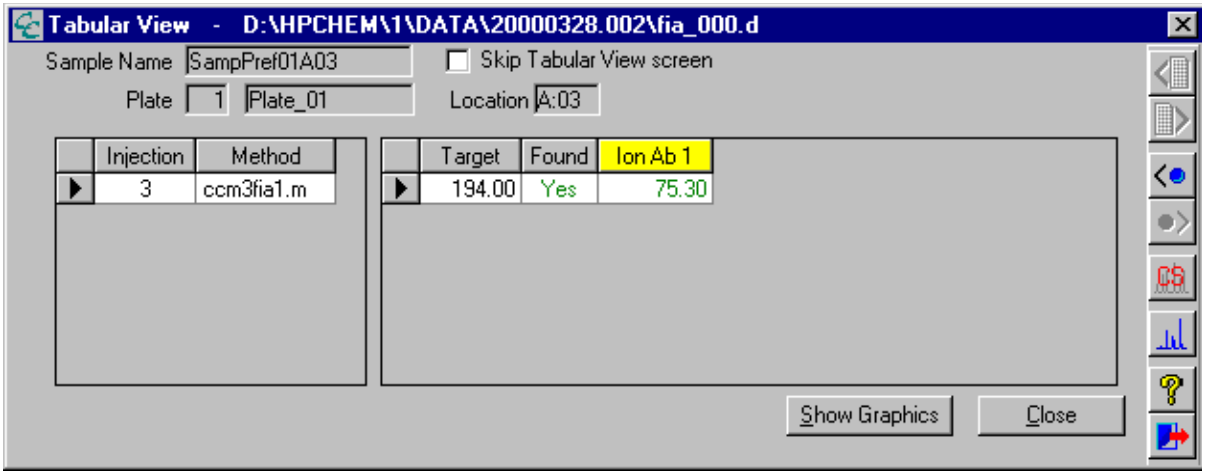
- Red = no target masses found
- Yellow = some target masses found
- Green = all target masses found



To view the results of a study for individual samples

1 **Click on the View results toolbar button if it is not already selected.**

2 **Double-click on a sample well to see the Tabular View dialog box shown below.**



3 **To view data in other wells in this study, use the navigation buttons to:**

Return to the previous plate in the study.

Advance to the next plate in the study.

Return to the previous well in the plate.

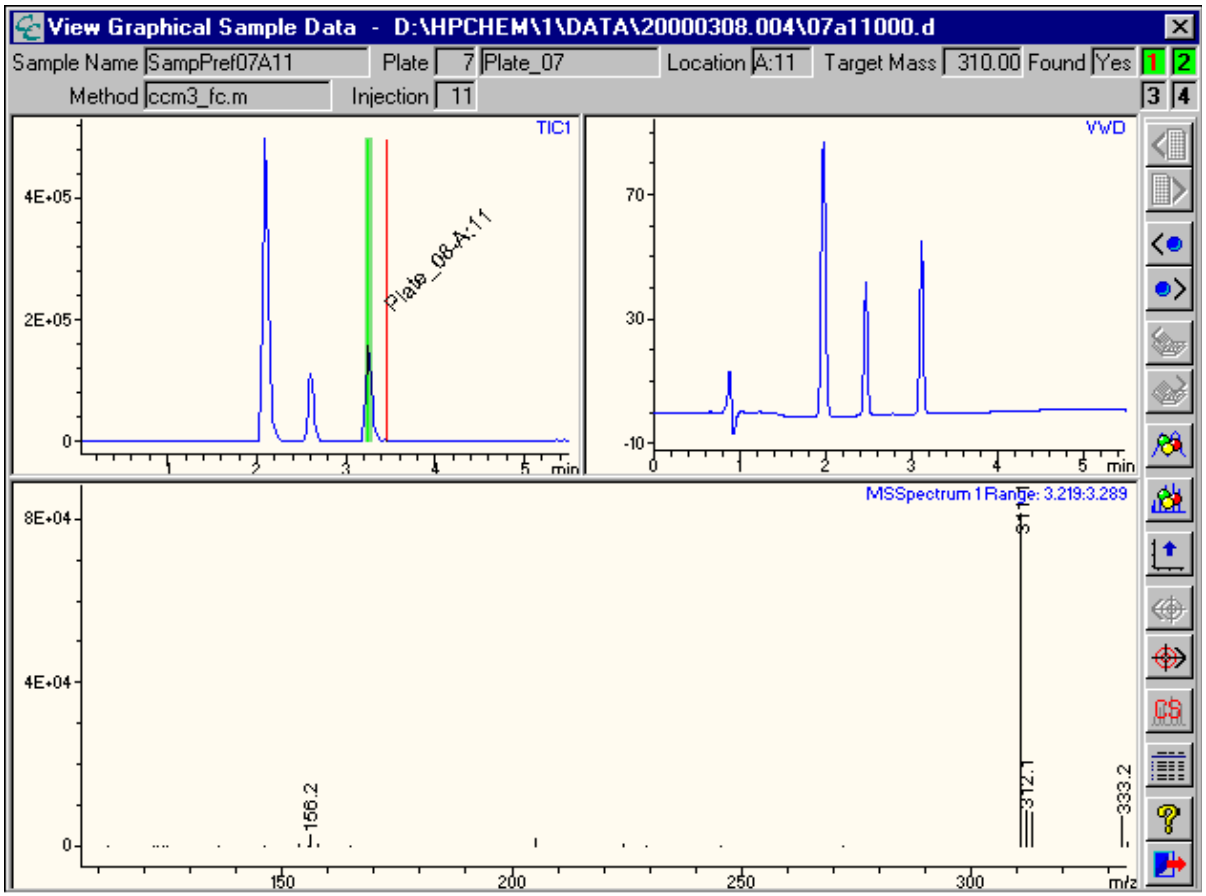
Advance to the next well in the plate.

4 **To view the data in more detail, click the CS button.**

This will load the data file into ChemStation and put ChemStation in to the View Data mode. The CCMode screen will be minimized.

5 **To view graphical data for the sample, click either the chromatogram or Show Graphics button to display the following dialog box.**

To view the results of a study for individual samples

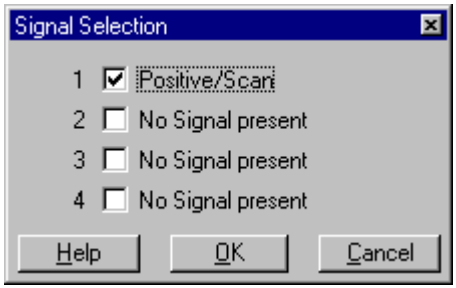


The upper right part shows the UV or other chromatogram
The lower part shows the mass spectrum, with the most abundant masses labeled.

If the data was acquired with the LC/MSD, a Signal selector/indicator box is located in the upper right corner of the screen.

Red numbers: The signal is selected for display.
Green background: The signal is present in the data file.
Black numbers: The signal is *not* selected for display.
Gray background: The signal is *not* present in the data file.

When you click inside of each of the signal selector indicators, the Signal selection dialog box appears.



You can select a different MSD signal for display if more than one signal was collected during data acquisiton, then click **OK**.

The toolbar at the right side of the View Graphical Sample data screen has the following buttons:

Toggles between Overlay and Separate TIC signals.

Toggles between Overlay and Separate Mass spectrum signals.

Toggles between all in the same Scale and each signal in Full Scale. This applies to TIC and Mass Spectrum signals simultaneously.

Return to the previous Target Mass in the Purity table. Clicking this button is equivalent to placing the pointer one row up in the Purity table from the Tabular view screen.

Advance to the next Target Mass in the Purity table. Clicking this button is equivalent to placing the pointer one row down in the Purity table from the Tabular view screen.

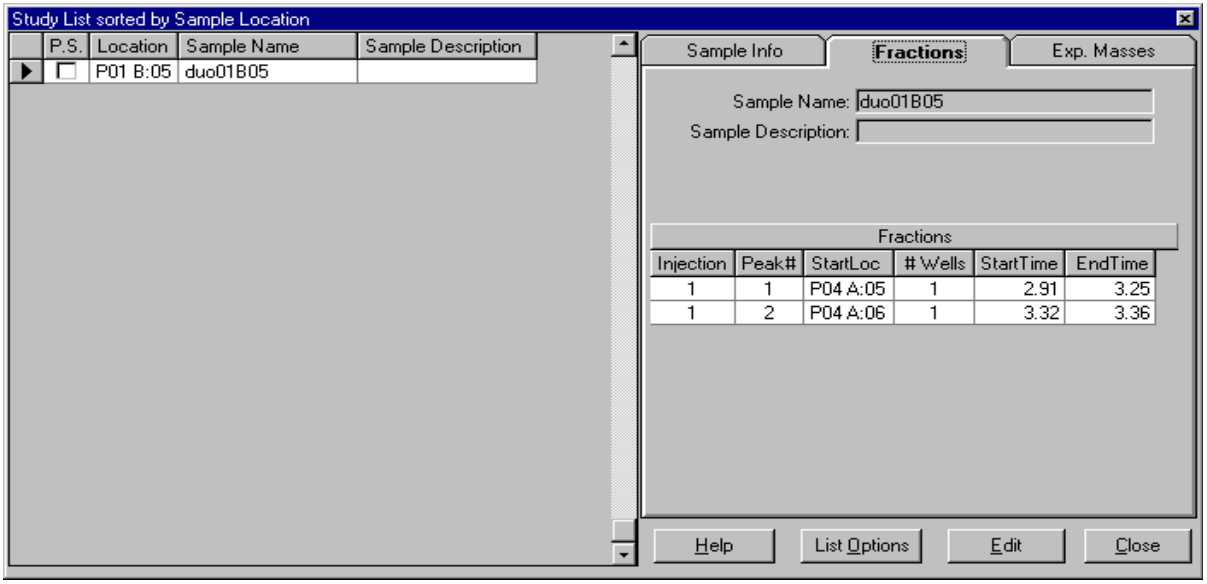
6 To view data from another method, use the method selection buttons to:

Return to the previous Method in the Method table.

Advance to the next Method in the Method table.

7 Click the Study lists button in the toolbar of the CC-Mode Main window to display the Study List sorted by Sample Location dialog box.

8 Click on the Fractions tab as shown below.



If the sample has fractions, the following information is displayed:

Injection Number indicating the injection. This number corresponds with the method list in the Sample Info Tab. When, for example, 3 methods are defined to run on this sample, the number will run from 1 to 3.

Peak # Indicates the peak within the run.

Start Loc. Shows the location within the microtiter plate or rack where this fraction begins.

 # Wells Indicates the number of wells (or tubes) this fraction occupies.

Start Time The Run Time when this fraction started collection.

End Time The Run Time when this fraction stopped collection.

To view the sample/fraction relation

1 **Click on the View results toolbar button if it is not already selected.**

2 **Click with the left mouse button on a sample well of interest.**

The sample well and the fraction well (if any), which were collected from this sample, will be marked with a dot.

Fraction information is displayed in the chromatogram:

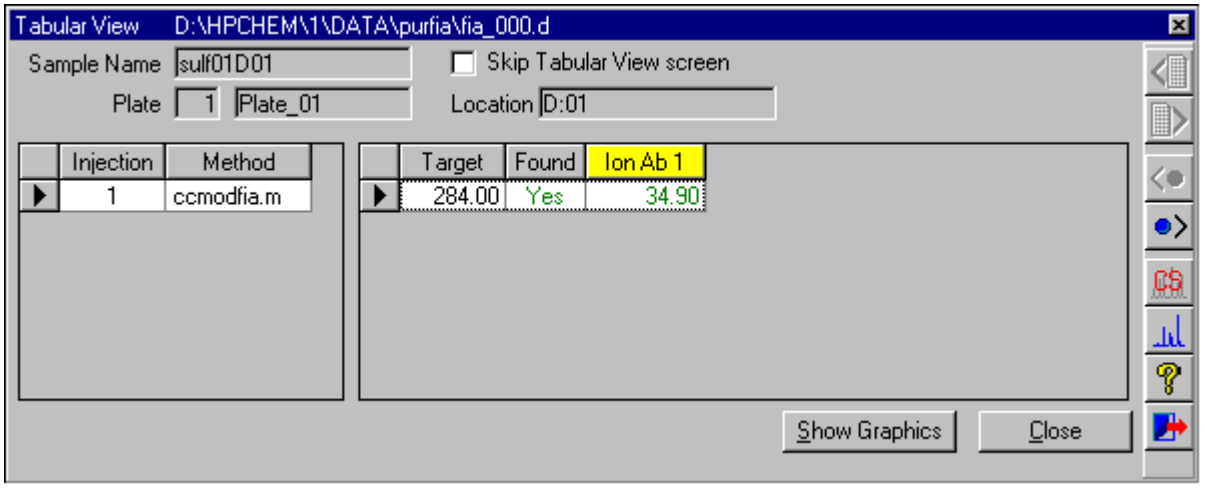
- A vertical green bar indicates the start of the fraction
- A vertical red bar indicates the end of the fraction
- The fraction coordinates are shown in a diagonal label (plate location)
- The threshold is shown as a dotted line

To review FIA results

In an FIA study, multiple injections are stored in one data file. The maximum number of injections is set by the FIA number of injections parameters on the Study Configuration screen.

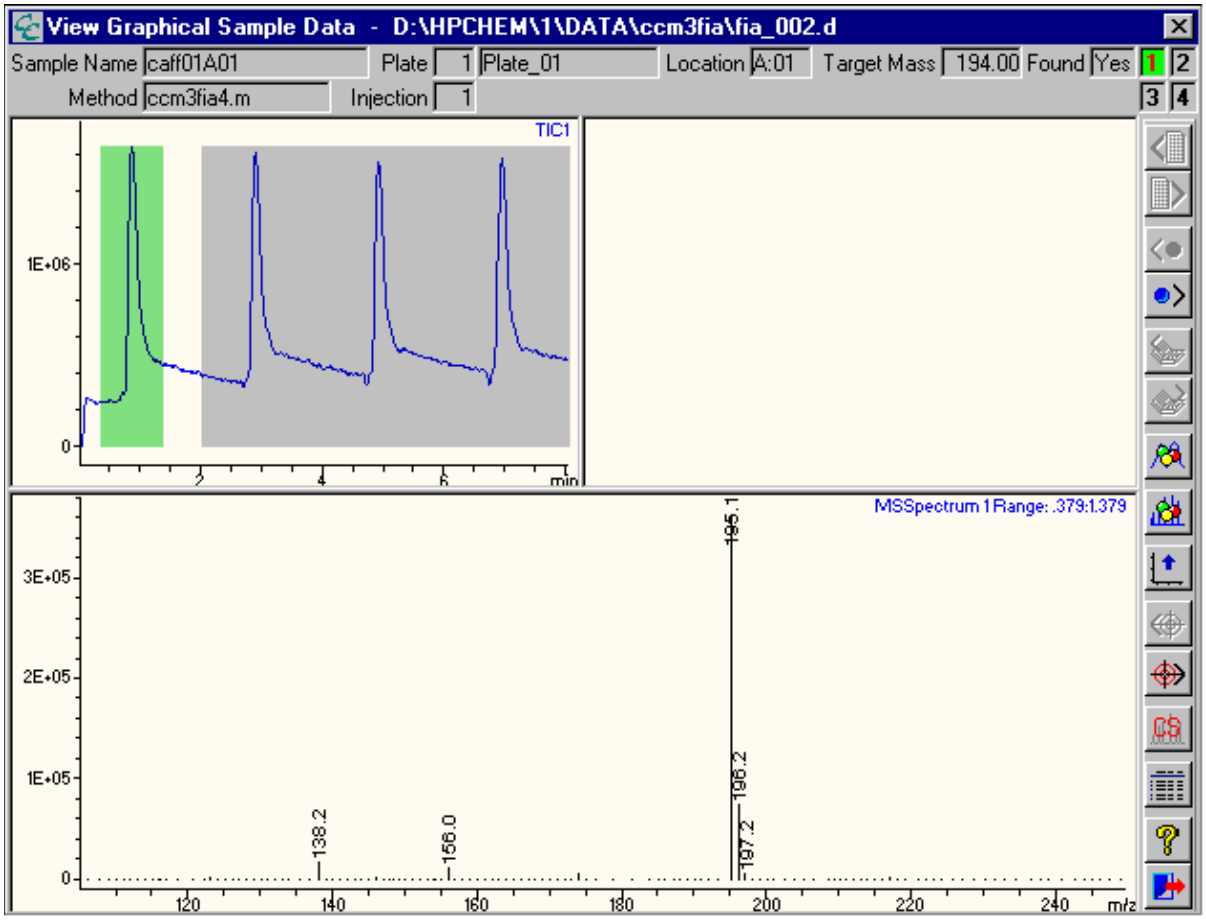
1 **Click on the View results toolbar button if it is not already selected.**

2 **Double-click on a sample well to see the Tabular View dialog box shown below.**



3 **To view graphical data for the run, click Show Graphics to display the following dialog box.**

The peak highlighted in green on the total ion chromatogram (TIC) in the upper half of the window is the current well position. The mass spectrum for the current sample is displayed in the lower half of the window. As you navigate from one well position to another using the navigation buttons on the toolbar at the right side of the window, the relevant peak will be highlighted and the corresponding mass spectrum displayed.


View Graphical Sample Data - D:\HPCHEM\1\DATA\ccm3fia\fia_002.d
Sample Name caff01A01
Plate 1 Plate_01
Location A:01
Target Mass 194.00
Found Yes
Method ccm3fia4.m
Injection 1
TIC1
1E+06
0
min
MSSpectrum 1 Range: .379:1.379
3E+05
2E+05
1E+05
0
195.1
196.2
197.2
138.2
156.0
120
140
160
180
200
220
240
m/z

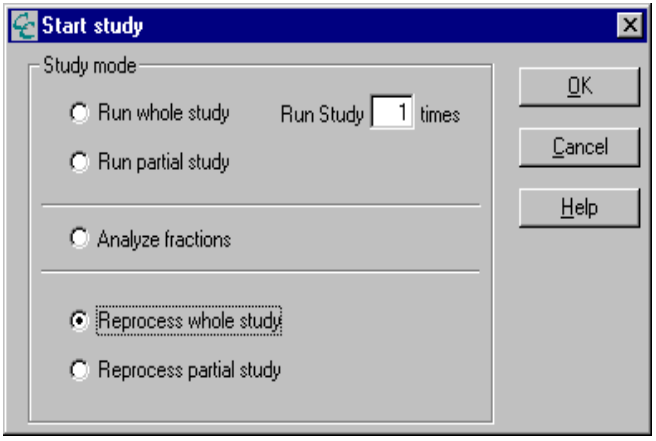
To reprocess a study

Reprocessing allows you to use different data analysis parameters to process the analytical data already acquired for a study.

1 **Change any parameters you wish, such as integration parameters or methods.**

2 **If you only want to reprocess some samples, select P.S. in the Study list next to each sample of interest.**

3 **Click on the Start Study toolbar button.**



4 **Select Reprocess whole study, then click OK.**

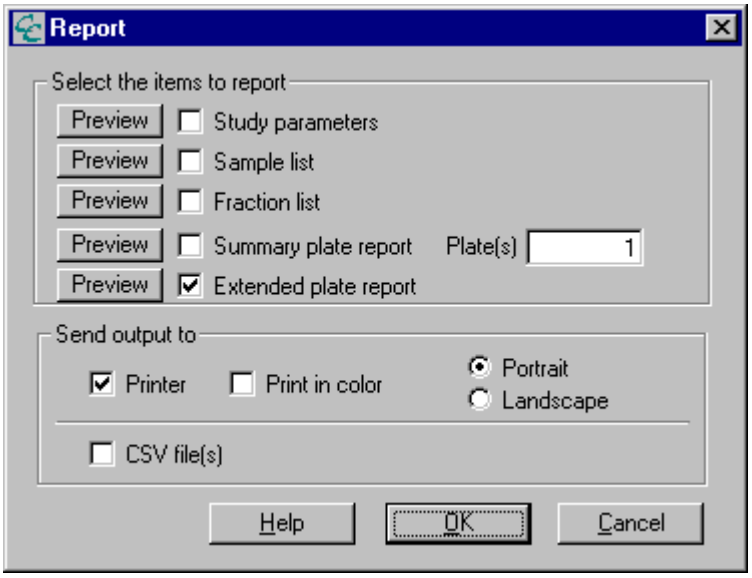
To only view the results for samples selected for Partial study (P.S.) in the Study list, select **Reprocess partial study**.

CAUTION Reprocessing studies changes settings in ChemStation method as described below.

If you have selected Disable ChemStation Report in the Specify Report screen, the report destination will be cleared in the ChemStation method. You will need to reselect the report destination in order to generate ChemStation reports.

To print or export results

1 Select Reporting **from the File menu to display the Report dialog box.**



2 Select the plate number and the items to report.

Summary plate report

Prints a simple graphical report, showing the qualification result of each sample well. If multiple methods were used to analyze the samples, the results for each method are printed separate pages.

Extended plate report

Prints an extended plate report, which may contain the following types of information:

- Adduct/dimers/user-selected masses
- Background masses to eliminate
- Purity calculation parameters
- Ionization polarity
- Sample information, such as sample description, signal selection, purity, retention times, highest m/z

3 Select the output, then click OK.

Printer The selected report is sent to the default Windows printer.

CSV File You are prompted to select a directory and file name for export.
The default file name is export.csv in the Import file path specified in the System Configuration dialog box.

To review CC-Mode results remotely

CC-Mode results can be viewed on a computer other than the one used for acquiring sample data.

1 **Verify that the other computer has Windows NT 4.0, LC and LC/MSD ChemStation software, and CC-Mode software installed (options INB22004 and INB22002 LC/MSD Data Analysis).**

Be sure to comply with the terms of the license agreements for all software.

2 **From the computer with the data, you will need to use the following files:**

- Any methods (*.m) used for your study. These need to be placed in the directory specified in the CC-Mode System Configuration screen
- All the data files (*.d) you wish to view. These files will be in subdirectories specified in the Study parameters screen.
- Copy the *.mdb file for your study. Verify that the file attribute is not “read only”. The source is the directory specified by the CCMode System Configuration screen on the system where the data was acquired.

3 **Use System Configuration in CC-Mode on the review computer to select the correct locations for the different files (studies, data, methods, import files).**

If the directory paths are not correct, the CC-Mode software cannot access the data.

4 **Use the Change data path button in the Study parameters screen to select the current data path.**

To review CC-Mode results remotely

9

System Checkout, 140
UV-triggered Fraction Collection Checkout, 153
Mass-based Fraction Collection Checkout, 163

Checkout Procedures

Describes how to perform the checkout procedure for the CC-Mode software and 220 micro plate sampler system, including fraction collection

Checkout Procedures

This chapter describes three different checkout procedures. These procedures are used to verify operation of the Agilent CC-Mode software and its associated hardware components and accessories.

System checkout procedure, page 140

This checkout procedure verifies operation of the Agilent 220 Micro Plate Sampler. It also verifies purity calculations, if INB22002 software is installed. The checkout procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 1100 Series LC/MSD (optional)
- Preparing the Agilent 220 Micro Plate Sampler
- Setting the study parameters, including purity calculation settings
- Starting the analysis
- Reporting the results

UV-triggered fraction collection checkout procedure, page 153

This checkout procedure verifies operation of the Agilent G2251A UV-fraction collection accessory used with the Agilent CC-Mode software. The checkout procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 220 Micro Plate Sampler
- Setting the study parameters
- Starting the analysis and collecting fractions

Mass-based fraction collection checkout procedure, page 163

This checkout procedure verifies operation of the Agilent G1968A mass-based fraction collection accessory used with the Agilent CC-Mode software. The checkout procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 220 Micro Plate Sampler
- Determining the fraction collection delay time
- Setting the study parameters
- Starting the analysis and collecting fractions
- Reanalysing the fractions

System Checkout

This section describes the system checkout procedure for the Agilent 1100 Series CC-Mode system. The checkout procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 1100 Series LC/MSD (optional)
- Preparing the Agilent 220 Micro Plate Sampler
- Setting the study parameters
- Starting the analysis
- Reporting the results

What You Will Need

☐ **Instruments**

- ☐ Agilent 1100 Series binary or quaternary pump with on-line degasser
- ☐ UV detector
- ☐ Agilent 220 Micro Plate Sampler
- ☐ Agilent G2251A fraction collection accessory (optional)
- ☐ Agilent 1100 Series LC/MSD (optional)

- ☐ **Software**
  - ☐ INB 22000 (part of G2250A) CC-Mode software for the Agilent ChemStation
  - ☐ INB 22002 CC-Mode Data Analysis software, license only (optional)
- ☐ **Column**
  - ☐ 4.6 mm x 15 cm Zorbax Eclipse XDB-C18 (Agilent part number 993967-902, holder 5021-1845)
- ☐ **Solvents (HPLC grade)**
  - ☐ bi-distilled water
  - ☐ acetonitrile
  - ☐ glacial acetic acid
- ☐ **Sample**
  - ☐ caffeine (Agilent part number 5063-6524)

Step 1 - Prepare the LC system

1 **Fill the solvent bottles with HPLC-grade bi-distilled water and 0.1% acetic acid (channel A) and acetonitrile (channel B).**

2 **Turn on the detector lamp and pump using the On/Off button in the Analysis screen.**

3 **Turn on the degasser by pressing the line-power switch.**

4 **Purge channel A and B of the pump.**

5 **Enter the method parameters as shown in Table 1.**

6 **Save this method with the name caffeine.m.**

7 **Allow the detector at least 15 minutes to provide a stable baseline.**

8 **Load caffeine.m from the ChemStation Method & Run Control screen.**

9 **Pump the water/acetonitrile (85/15%) mobile phase through the column for 10 minutes at a flow rate of 1.5 ml/min.**

Table 1 **Method Parameters (LC Only)**

Parameter	Value
Flow rate	0.8 ml/min
Channel A (water with 0.1% acetic acid)	85%
Channel B (acetonitrile)	15%
STOP time	3.5 minutes
Column temperature	40 °C
Wavelength (DAD)	272/10 nm, 360/100 nm
Wavelength (VWD)	272 nm

Table 2 **Method Parameters (LC/MSD)**

Parameter	Value
Flow rate	0.8 ml/min
Channel A (water with 0.1% acetic acid)	85%
Channel B (acetonitrile)	15%
STOP time	3.5 minutes
Column temperature	40 °C
Ion mode	Positive
Vcap	4000 V
Nebulizer	50 psig
Drying gas flow (APCI)	5 L/min
Drying gas flow (ESI)	12 L/min
Drying gas temp	325 °C
Corona (APCI)	4 μA
Vaporizer APCI)	400 °C
Scan range	100 - 300 amu
Stepsize	0.1
Peakwidth	0.12 min
Time filter	on
Fragmentor	80 V

Step 2 - Prepare the Agilent 220 micro plate sampler

1 **Install the 505H rack directly next to the dilutor.**

2 **Place a micro-plate in position 1 on the 220 micro plate sampler (see plate layout on the Study Layout screen in the CC-Mode software). Fill the contents of the caffeine sample concentration 0.1 mg/ml into Plate 1 well A 01.**

3 **Select Maintenance Mode from the Sampler menu in the CC-Mode main window.**

4 **Click the Continuous button to prime the tubing and needle to remove any air bubbles.**

5 **Click Stop when the tubing and syringe are free of air bubbles. This normally takes about 10 minutes.**

Step 3 - Create a new study

1 **Click the new study button.**

2 **Give the new study the name Caffeine.**

3 **Click OK.**

Step 4 - Set study parameters

1 **Open the Study Parameter screen.**

2 **Enter “test” in the Name Prefix field.**

3 **Enter your name in the Operator field.**

4 **Select ChemStation method caffeine.m.**

5 **Click the Edit Method button to set the sampler parameters shown in Table 3 for LC only or LC/MSD.**

Table 3 **Sampler Parameter Settings**

Task	Task Parameter	Setting
Rinse Needle Inside	Volume	100 µl
	Flow	3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min
Rinse Injection Port	Volume	100 µl
	Flow	3 ml/min
Create Air Gap	Volume	2 µl
Draw Sample	Sample Volume	20 µl for 5 µl sample loop; or 80 µl for 20µl sample loop
	Sample Draw Flow	0.3 ml/min
Wash Vial	Duration	1 s
Inject Sample	Flow	0.3 ml/min

Purity calculation settings (LC/MSD)

➤ Set the purity calculation parameters as shown in Table 4.

Table 4 **Purity Calculation Parameter Settings**

Area	Parameter	Setting
Adduct ions	M + H(1)	selected
	M + Na(23)	selected
Purity calculation	Enable purity calculation	selected
	Chromatographic separation	selected
Calculation used for qualification	TIC peak area	selected
	Qualifier level	10%
Chromatographic calculation	TIC peak area	selected
	TIC peak height	selected
	UV peak area	selected
	UV peak height	selected
Noise threshold	Percent of highest peak	5

Study layout

1 Open the Study Layout screen.

2 Select the sample in Plate 1, well A01 by clicking on the sample well with the left mouse button.

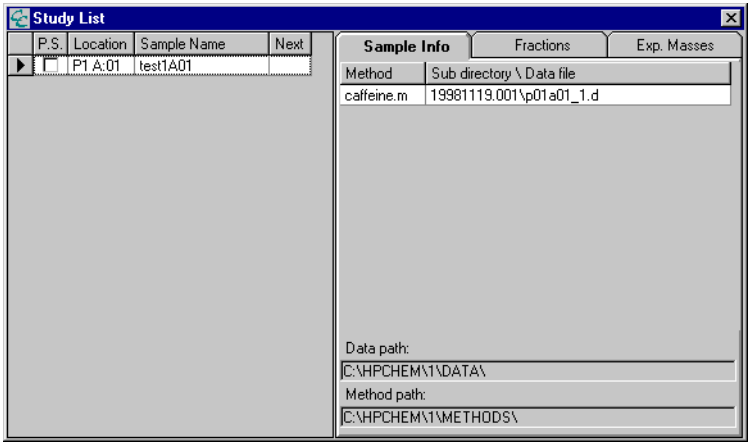
The edge of the selected well will turn white on the screen.

3 Add the sample to the study.

Click the right mouse button to display a pop menu that allows you to add the selected sample to the study.

Study list

1 Access the Sample Info tab in the Study List screen.



2 Note the sample name you selected.

When the Study List sequence is being generated from the selected sample after the run, the sample is named automatically according to the following format as selected in the Study parameters screen:

<prefix><plate number><well position>.

In this example, the sample name would be “test1A01”.

3 For data analysis, enter the following information in the Exp. masses tab:

Mass:194.08, or Formula:C8H10N402.

Step 5 - Run the analysis

Before you start

➤ Access the Sample Info screen in the ChemStation software and verify that the Data File is set to Manual and that the Vial is set to 1.

Start the analysis

1 **Start the analysis by clicking the green traffic light** **toolbar button.**

2 **Select Run whole study from the Start Study dialog box.**

3 **Click OK.**

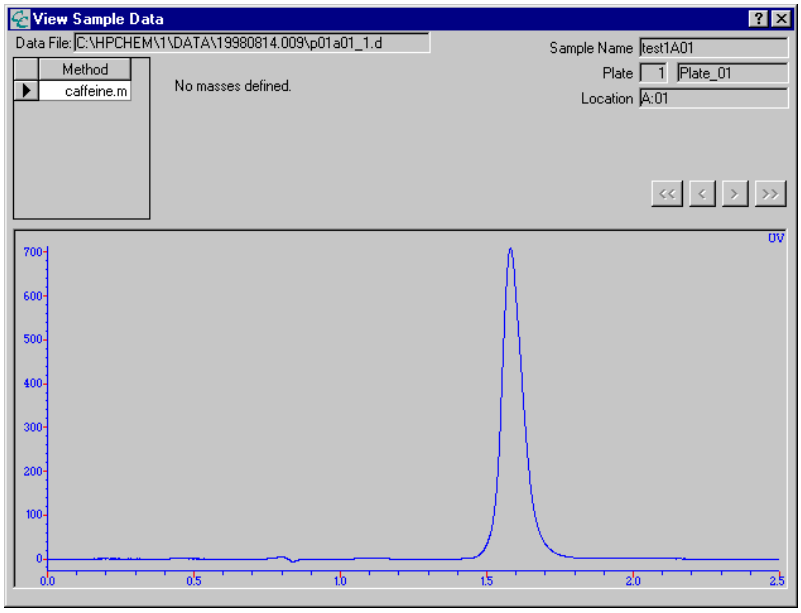
Check the system status

➤ Click the button on the Toolbar to display the Status screen.

This screen shows you what is happening with the ChemStation and the 220 micro plate sampler while the system is running.

Observe the chromatogram

➤ Double-click on the well containing the sample to display the chromatogram.



The Status bar shows the coordinates, sample name and data file name of the selected sample, the sample will be highlighted and the corresponding chromatogram appears.

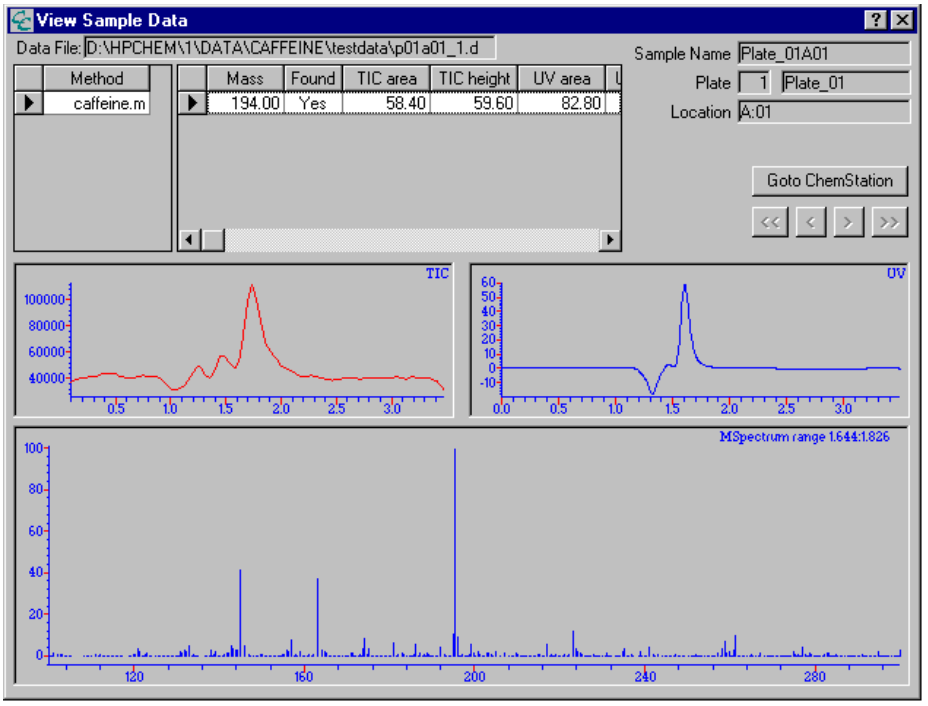
The exact profile of the chromatogram will depend on the column you have used. Differences in retention times of the peaks in your chromatogram and the one shown in the above figure might be a result of variations in the concentration of the sample from batch to batch, the quality of the solvents used and the column temperature.

Step 6 - Review LC/MSD results

1 **Be sure that the View Data toolbar button is selected.**

2 **Double-click on the well containing the sample.**

The View Sample Data window shows the sample information, the TIC, and mass spectrum. UV data will also be displayed if it was acquired.



UV-triggered Fraction Collection Checkout

This section describes the checkout procedure for the Agilent G2251A UV-fraction collection accessory used with the Agilent 1100 Series CC-Mode system. The checkout procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 220 Micro Plate Sampler
- Setting the study parameters
- Starting the analysis and collecting fractions

What You Will Need

☐ **Instruments**

- ☐ Agilent 1100 Series binary or quaternary pump with on-line degasser
- ☐ UV detector
- ☐ Agilent 220 Micro Plate Sampler
- ☐ Agilent G2251A fraction collection accessory

- ☐ **Software**
  - ☐ INB 22000 CC-Mode software for the Agilent ChemStation
- ☐ **Column**
  - ☐ 4.6 mm x 15 cm Zorbax Eclipse XDB-C18 (Agilent part number 993967-902, holder 5021-1845)
- ☐ **Solvents**
  - ☐ bi-distilled water
  - ☐ acetonitrile
  - ☐ glacial acetic acid
- ☐ **Sample**
  - ☐ caffeine (Agilent part number 5063-6524)

Step 1 - Prepare the LC system

1 **Fill the solvent bottles with HPLC-grade bi-distilled water and 0.1% acetic acid (channel A) and acetonitrile (channel B).**

2 **Turn on the detector lamp and pump using the On/Off button in the Analysis screen.**

3 **Turn on the degasser by pressing the line-power switch.**

4 **Purge channels A and B of the pump.**

5 **Enter the LC method parameters as shown in Table 5.**

6 **Save this method with the name uvfract.m.**

7 **Allow the detector at least 15 minutes to provide a stable baseline.**

8 **Load caffeine.m from the ChemStation Method & Run Control screen.**

9 **Pump the water/acetonitrile (85%/15%) mobile phase through the column for 10 minutes at a flow rate of 1.5 ml/min.**

Table 5 **LC Method Parameters for UV fraction collection checkout**

Parameter	Value
Flow rate	1.5 ml/min
Channel A (water with 0.1% acetic acid)	85%
Channel B (acetonitrile)	15%
STOP time	2.5 minutes
Column temperature	40 °C
Wavelength (DAD)	272/10 nm, 360/100 nm
Wavelength (VWD)	272 nm

Step 2 - Prepare the Agilent 220 micro plate sampler

1 **Install the 505H rack directly next to the dilutor.**

2 **Place a micro-plates in positions 1 and 2 on the 220 micro plate sampler (see plate layout on the Study Layout screen in the CC-Mode software). Fill the contents of the diluted caffeine sample concentration 0.1 mg/ml into Plate 1 well A 01.**

3 **Select Maintenance Mode from the Sampler menu in the CC-Mode main window.**

4 **Click the Continuous button to prime the tubing and needle to remove any air bubbles.**

5 **Click Stop when the tubing and syringe are free of air bubbles. This normally takes about 10 minutes.**

Step 3 - Create a new study

1 **Click the new study button.**

2 **Give the new study the name UV Fraction Collection.**

3 **Click OK.**

Step 4 - Set study parameters

1 **Open the Study Parameter screen.**

2 **Enter “test” in the Name Prefix field.**

3 **Enter your name in the Operator field.**

4 **Select Use next vial for Overfill Action, and Follow well numbers for Fill Order.**

5 **Select ChemStation method uvfract.m.**

6 **In Study Layout section, click Plate 2 Type and change to Fractions. Make sure the Plate 1 Type is “Samples”.**

Use the default values for other parameters.

7 **Click the Edit Method button to set the sampler parameters shown in Table 6.**

Table 6 **Sampler Parameter Settings**

Task	Task Parameter	Setting
Rinse Needle Inside	Volume	100 µl
	Flow	3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min
Rinse Injection Port	Volume	100 µl
	Flow	3 ml/min
Create Air Gap	Volume	2 µl
Draw Sample	Sample Volume	20 µl for 5 µl sample loop; or 80 µl for 20µl sample loop
	Sample Draw Flow	0.3 ml/min
Wash Vial	Duration	1 s
Enable Fraction Collection	Time	0.00
	Event	Peak Detection
	Signal Source	DAD, FLD, VWD or ADC
	Threshold	5 mAu (modify as appropriate)
	Peak Slope	5
	Maximum Fraction Duration	1 min
Inject Sample	Flow	0.3 ml/min

Study layout

1 **Open the Study Layout screen.**

2 **Select the sample in Plate 1, well A01 by clicking on the sample well with the left mouse button.**

The edge of the selected well will turn white on the screen.

3 **Add the sample to the study.**

Click the right mouse button to display a pop menu that allows you to add the selected sample to the study.

Step 5 - Run the analysis

Before you start

➤ Access the Sample Info screen in the ChemStation software and verify that the Data File is set to Manual and that the Vial is set to 1.

Start the analysis

1 **Start the analysis by clicking the green traffic light** **toolbar button.**

2 **Select** Run whole study **from the Start Study dialog box.**

3 **Click** OK**.**

4 **Verify that the sampler delivers the expected fraction into Plate 2.**

There should be only one fraction, but it may be diverted into more than one well. If no fractions or multiple fractions are collected, raise or lower the peak detection threshold under Enable Fraction Collections in the Sampler Parameter settings screen and rerun the study.

Mass-based Fraction Collection Checkout

This section describes the checkout procedure for the Agilent G1968A mass-based fraction collection used with the Agilent 1100 Series CC-Mode system. The check-out procedure consists of the following activities:

- Preparing the Agilent 1100 Series LC
- Preparing the Agilent 220 Micro Plate Sampler
- Determining the fraction collection delay time
- Setting the study parameters
- Starting the analysis and collecting fractions
- Reanalysing the fractions

What You Will Need

☐ **Instruments**

- ☐ Agilent 1100 Series binary or quaternary pump with on-line degasser
- ☐ Agilent 1100 Series LC/MSD
- ☐ Agilent 220 Micro Plate Sampler
- ☐ Agilent G1968A mass-based fraction collection accessory, which includes Agilent G2251A fraction collection accessory

☐ **Software**

- ☐ INB 22000 CC-Mode software for the Agilent ChemStation
- ☐ INB 22002 CC-Mode Data Analysis software
- ☐ INB 22003 mass-based fraction collection software for the Agilent ChemStation

☐ **Column**

- ☐ 4.6 mm x 15 cm Zorbax Eclipse XDB-C18 (Agilent part number 993967-902, holder 5021-1845)

☐ **Solvents**

- ☐ bi-distilled water
- ☐ acetonitrile
- ☐ glacial acetic acid

☐ **Sample**

- ☐ Delay calibrant (Agilent part number G1946-85020)
- ☐ Electrospray LC demo sample (Agilent part number 59987-20033)

Step 1 - Prepare the LC system

1 **Fill the solvent bottles with HPLC-grade bi-distilled water and 0.1% acetic acid (channel A) and acetonitrile (channel B).**

2 **Turn on the pump using the On/Off button in the Analysis screen.**

3 **Turn on the degasser by pressing the line-power switch.**

4 **Purge channels A and B of the pump.**
No column should be installed at this time.

Step 2 - Determine the collection delay time

Before running the mass-based fraction collection, the collection delay time must be determined. The collection delay time is the difference in time between when the sample arrives at the MSD and when it arrives at the fraction collector.

The delay sensor and I/O accessory module and cabling should already be installed as per the instructions in the “Agilent 220 Micro Plate Sampler Reference Manual”.

1 **If it has not already loaded, copy the fcdelay.m method from the disk supplied in the mass-based fraction collection kit to the \hpchem\1\methods directory on the ChemStation.**

Make sure that the method and all the files in its directory are read-write. Copy the **fcd_pre.mac** and **fcd_post.mac** macro files from the disk to the \hpchem\core directory.

2 **Load the fcdelay.m method and verify that the method parameters match those given in Table 7.**

Table 7 **Method Parameters for fraction collection delay**

Parameter	Value
Pre-run Cmd/Macro	macro "fcd_pre.mac",go
Data Acquisition	On
Standard Data Analysis	On
Post-Run Cmd/Macro	macro "fcd_post.mac",go
LC Flow rate	2.0 ml/min
Channel A (water with 0.1% acetic acid)	50%
Channel B (acetonitrile)	50%

Table 7 **Method Parameters for fraction collection delay**

Parameter	Value
STOP time	1.0 minute
Wavelength (DAD)	550/10 nm
Wavelength (VWD)	595 nm
MSD Ion Mode	Positive
VCap	3500 V
Nebulizer	25 psig
Drying gas flow (APCI)	5 l/min
Drying gas flow (ES)	12 l/min
Drying gas temp	350 degrees C
Corona (APCI)	4 uA
Vaporizer (APCI)	400 degrees C
Scan range	350 - 450 amu
Step size	0.1
Fragmentor	70
Gain	1.0
Threshold	50
Peak width	0.1 min
Time filter	disabled
Data storage	Full

Step 3 - Prepare the 220 micro plate sampler

1 **Install the 505H rack directly next to the dilutor.**

2 **Place a micro-plate in position 1 on the 220 micro plate sampler (see plate layout on software Study Layout screen).**

3 **Fill Plate 1 well A 01 with the green delay calibrant.**

4 **Select Maintenance Mode from the Sampler menu in the CC-Mode main window.**

5 **Click the Continuous button to prime the tubing and needle to remove any air bubbles.**

6 **Click Stop when the tubing and syringe are free of air bubbles. This normally takes about 10 minutes.**

Step 4 - Create a new study

1 **Click the new study button.**

2 **Give the new study the name Delay Calibration.**

3 **Click OK.**

Step 5 - Set study parameters

1 **Open the Study Parameter screen.**

2 **Enter “test” in the Name Prefix field.**

3 **Enter your name in the Operator field.**

4 **Select ChemStation method fcdelay.m.**
Use the default values for other parameters.

5 **Click the Edit Method button to set the sampler parameters shown in Table 8.**

Table 8 **Sampler Parameter Settings**

Task	Task Parameter	Setting
Rinse Needle Inside	Volume	100 µl
	Flow	3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min
Rinse Injection Port	Volume	100 µl
	Flow	3 ml/min
Create Air Gap	Volume	2 µl
Draw Sample	Sample Volume	20 µl for 5 µl sample loop; or 80 µl for 20µl sample loop
	Sample Draw Flow	0.3 ml/min
Wash Vial	Duration	1 s
Inject Sample	Flow	0.3 ml/min
Enable Fraction Coll. Delay Sensor		

Study layout

1 **Open the Study Layout screen.**

2 **Select the sample in Plate 1, well A01 by clicking on the sample well with the left mouse button.**

The edge of the selected well will turn white on the screen.

3 **Add the sample to the study.**

Click the right mouse button to display a pop menu that allows you to add the selected sample to the study.

Step 6 - Run the analysis

Before you start

➤ Access the Sample Info screen in the ChemStation software and verify that the Data File is set to Manual and that the Vial is set to 1.

Start the analysis

1 **Start the analysis by clicking the green traffic light** **toolbar button.**

2 **Select** Run whole study **from the Start Study dialog box.**

3 **Click** OK.

When the run has completed, a report will be printed which will display the MSD TIC, the UV signal, and the MSD delay sensor signal. The collector delay setting for the MSD will be displayed in the lower part of the report. You will need to enter this value later as “Collector Delay” in the fraction collection parameters screen in the ChemStation method.

Step 7 - Set up mass-based fraction collection method

1 **Install the 4.6 mm x 15 cm Zorbax Eclipse XDB-C18 column**

2 **Enter the method parameters as shown in Table 9.**

3 **Save this method to msfract1.m, and then save it a second time to msfract2.m.**

4 **Pump the water/acetonitrile (80%/20%) mobile phase through the column for 10 minutes at a flow rate of 2.0 ml/min.**

Table 9 **Method Parameters for mass-based fraction collection**

Parameter	Value
LC Flow rate	2.0 ml/min
Channel A (water with 0.1% acetic acid)	80%
Channel B (acetonitrile)	20%
Timetable	3.5 min Channel A = 40%Channel B = 60%
STOP time	5.5 minutes
Post time	2 minutes
Column temperature	30 degrees C
MSD Ion Mode	Positive
VCap	2500 V
Nebulizer	35 psig
Drying gas flow (APCI)	5 l/min
Drying gas flow (ES)	12 l/min
Drying gas temp	350 degrees C
Corona (APCI)	4 uA
Vaporizer (APCI)	400 degrees C
Scan range	100 - 350 amu
Step size	0.1
Fragmentor	60
Gain	1.0
Threshold	100

Table 9 **Method Parameters for mass-based fraction collection**

Parameter	Value
Peak width	0.1 min
Time filter	on
Data storage	Condensed
Fraction Collection Mode	Use method info
Detector	MS only
Signal 1	Used
Positive Adducts	+H
Base Mass	278.0, 284.0, 310.0
Model Type	Other
Trigger Type	Negative step
Min Peak Width	0 min
Max Peak Width	0.5 min
Time Limit	1.0 min
MSD m/z detection - peak slope	500
Collector Delay	(determine from FC delay test)
Threshold	2000 (modify as appropriate)
Mass Window	0.5
Analog Output Range	2000

Step 8 - Prepare the 220 micro plate sampler

1 **Install the 505H rack directly next to the dilutor.**

2 **Place micro-plates in position 1 and 2 on the 220 micro plate sampler (see plate layout on software Study Layout screen).**

3 **Fill Plate 1 well A 01 with the Electrospray LC Demo sample.**

4 **Select Maintenance Mode from the Sampler menu in the CC-Mode main window.**

5 **Click the Continuous button to prime the tubing and needle to remove any air bubbles.**

6 **Click Stop when the tubing and syringe are free of air bubbles. This normally takes about 10 minutes.**

Step 9 - Create a new study

1 **Click the new study button.**

2 **Give the new study the name MS Fraction Collection.**

3 **Click OK.**

Step 10 - Set study parameters

1 **Open the Study Parameter screen.**

2 **Enter “test” in the Name Prefix field.**

3 **Enter your name in the Operator field.**

4 **Select Use next vial for Overfill Action, and Follow well numbers for Fill Order.**

5 **Under Fraction analysis, select Enable automatic analysis, then select msfract2.m for the Method for fraction analysis.**

6 **Under Method, select ChemStation method msfract2.m (you will need to edit the sampler parameters for this method first).**

7 **In Study Layout section, click Plate 2 Type and change to Fractions. Plate 1 Type should be listed as Samples.**

Use the default values for other parameters.

8 **Click the Edit Method button to set the sampler parameters shown in Table 10.**

Table 10 **Sampler Parameter Settings for msfrac2.m method**

Task	Task Parameter	Setting
Rinse Needle Inside	Volume	100 µl
	Flow	3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min
Rinse Injection Port	Volume	100 µl
	Flow	3 ml/min
Create Air Gap	Volume	2 µl
Draw Sample	Sample Volume	20 µl for 5 µl sample loop; or 80 µl for 20µl sample loop
	Sample Draw Flow	0.3 ml/min
Wash Vial	Duration	1 s
Inject Sample	Flow	0.3 ml/min

9 Under Method, select msfract2.m and change it to ChemStation method msfract1.m.

This is the method that you will use to inject the Electrospray LC Demo sample.

10 Click the Edit method button to set the sampler parameters as shown in Table 12.

Table 11 **Sampler Parameter Settings for msfract1.m method**

Task	Task Parameter	Setting
Rinse Needle Inside	Volume	100 µl
	Flow	3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min
Rinse Injection Port	Volume	100 µl
	Flow	3 ml/min
Create Air Gap	Volume	2 µl
Draw Sample	Sample Volume	20 µl for 5 µl sample loop; or 80 µl for 20µl sample loop
	Sample Draw Flow	0.3 ml/min
Wash Vial	Duration	1 s
Enable Fraction Collection	Based on masses	on
Wash Needle After Fraction	Volume	100 µl
	Flow	3 ml/min
Inject Sample	Flow	0.3 ml/min
Rinse Needle Outside	Volume	100 µl
	Flow	3 ml/min

Study layout

1 **Open the Study Layout screen.**

2 **Select the sample in Plate 1, well A01 by clicking on the sample well with the left mouse button.**

The edge of the selected well will turn white on the screen.

3 **Add the sample to the study.**

Click the right mouse button to display a pop menu that allows you to add the selected sample to the study.

Step 11 - Run the analysis

Before you start

➤ Access the Sample Info screen in the ChemStation software and verify that the Data File is set to Manual and that the Vial is set to 1.

Start the analysis

1 **Start the analysis by clicking the green traffic light** **toolbar button.**

2 **Select** Run whole study **from the Start Study dialog box.**

3 **Click** OK.

The analysis should generate three peaks (the first two of four compounds coelute). After the sample run has completed, the system will automatically inject the fractions that were collected.

4 **Verify that the system collects the expected fractions into Plate 2.**

If no fractions or too many fractions are collected, evaluate the EICs corresponding to masses 279, 285, and 311 in ChemStation Data Analysis to determine the proper threshold. Raise or lower the peak detection threshold in the Fraction Collection Parameters screen in the ChemStation method and rerun the study.

5 **Inject the collected fractions.**

Each fraction should contain a single peak at the retention time corresponding to the expected fraction.

Index

Symbols

.csv file extension, 51, 106
.mdb file extension, 38
.mth file extension, 40

A

Acetonitrile solvent, 141
Add Samples to Study dialog, 49
Adding fractions, 54
Adducts, selecting for purity calculations, 97
Analog to digital converter, 12, 22
  configuring, 94
Analyzing fractions, 119
  automatically, 120
Auto data subdirectory generation option, 46
Automatic analysis of fractions, 120

B

Background masses to subtract, 98

C

Caffein sample, 141
Calculate Mass dialog, 104
Calibrating tubing volume, 72
Cautions
  configuring ChemStation, 12
  reprocessing changes ChemStation methods, 132
  reprocessing changes ChemStation settings, 113
  starting CC-Mode software, 15
  testing customized plates, 27
  turning on ChemStation, 15
  wash needle after, 110
CC-Mode
  Base Software, 13
  configuring software, 16, 20
  data analysis software, 141, 164
  default software directory, 14
  installing software, 13
  Main window, 21
  menu items, 17
  optional software, 13
  parameters set in ChemStation, 112
  review results remotely, 135
  Sampler Only mode, 20
  Sampler/Fractionator mode, 20
  set up ChemStation method, 94
  software, 141
  software components, 13
  software installation, 13
  software loading, 16
  software operating modes, 20
  starting, 17
  starting software, 15
  starting software caution, 15
  system checkout procedures, 138, 140
  toolbar buttons, 17
  uninstalling software, 14
  warning during software installation, 14
Checkout
  column required, 141
  instruments required, 140
  sample required, 141
  software required, 141
  solvents required, 141
ChemStation, 17, 141
  acquiring data method, 57
  adding data methods, 57
  CC-Mode parameters, 112
  changes during reprocessing, 113
  configuring, 12
  data analysis report, enabling, 100

data file destination, 46
deleting data method, 57
editing method parameters, 58
ensuring correct path, 23
extended with CC-Mode software, 17
FIA method used with CC-Mode, 114
generating reports, 112
installing, 12
method directory, 40
method to use with CC-Mode, 94
methods of analysis list, 44
peak end time, 81
peak start time, 81
printing reports, 100
processing data method, 57
replacing data method, 57
reports, 46, 94
reports generated for FIA studies, 116
reprocessing studies, 94
selecting method, 57
setting up method for CC-Mode, 94
software, 13
Chromatograms
print in reports, 48
UV, 101
Chromatographic calculations, 98
Collection delay time
determining for mass-based fraction collection, 166
Communication (COM) port, default, 22
Configuring
CC-Mode software, 16, 20
ChemStation caution, 12
racks, 25
system, changing, 21
Create New Study dialog, 42
CSV files, 47
as import source, 52
configuring layout, 106
exported data, 134
importing expected masses from, 106
layout, 51
names, 48
overwrite existing, 48
path to storage, 48
Customized plates testing caution, 27

D

Data analysis
setting parameters, 95
standard, 113
Database for studies, 39
Default directory
CC-Mode software, 14
name limitations, 14
Detection, peak, 22, 80
parameters, 81
parameters disabled, 110
Detectors
DAD, VWD, FLD, 22
output, 79
supported, 22
Dimers, selecting for purity calculations, 97
Directory, installation
name limitations, 14

E

Edit Method dialog, 97
Enable Fraction Collection dialog, 109
export.csv file, 134
Extended Plate report, 133
Extracted ion chromatogram (EIC), 99

F

FIA
ion abundance calculations, 98
isocratic mobile phase conditions, 114
number of injections parameter, 130
using ChemStation method with CC-Mode, 114

Files
data, 23
import, 23
method, 23
paths, 23
Fill direction, 30
Fraction collection
accessory hardware, 12, 20
enable delay sensor, 83
installing module, 14
labels, 47
mass-based, 22, 109
mass-based process overview, 92
UV-based, 22
wash needle after caution, 110
Fraction Collection mode, 16
Fraction collection table
described, 79
duration (width), 79
events, 79
events detailed, 80
needle-cleaning schedule, 80
time, 79
Fraction collection, mass-based
column required, 164
creating new study, 169, 178
determining collection delay time, 166
instruments required, 163
method parameters, 175
method parameters for collection delay, 166
preparing LC system, 165
preparing micro plate sampler, 177
running the checkout analysis, 173
running the mass-based fraction collection analysis, 183
sample required, 164
sampler parameter settings, 180
setting study parameters, 170, 179
setting the method, 174
software required, 164
solvents required, 164
study layout, 171, 182
system checkout procedures, 163
Fraction collection, UV-triggered
column required, 154
creating new study, 158
instruments required, 153
running analysis, 162
sample required, 154
setting study parameters, 159
software required, 154
solvent required, 154
study layout, 161
system checkout procedure, 153
Fractions
analyzing, 119
analyzing automatically, 120
tab information displayed, 128

G

G2251A fraction collection hardware accessory, 14
Glacial acetic acid, 141
Graphical reports, 101
mass spectra, 101
total ion chromatogram, 101
UV chromatogram, 101

H

Hardware, optional
fraction collection accessory, 12
LC/MSD, 12
Hardware, required, 12
Homing, 32
Horizontal plots, 48

I

Import Options dialog, 51, 107
Importing
expected masses from CSV files, 106

from CSV file dialog, 52, 108
INB22000, 13, 20, 41
INB22001, 14
INB22002, 13, 20, 38, 135
INB22003, 13, 20, 81, 109
INB22004, 13, 135
Information
for study, 39
method related, 40
Injections
ordered by method option, 44, 96
standard program, 84
techniques, 89
Installing
CC-Mode software, 13
ChemStation, 12
optional software, 13
required hardware, 12
Ion abundances, 98
Isotopes, specifying for purity calculations, 97

L

LC
checkout methods parameters, 143
modules for analysis, 112
preparing for checkout, 142
preparing for mass-based fraction collection checkout, 165
preparing for UV-triggered fraction collection checkout, 155
setting sampler parameters, 147
UV-triggered fraction collection checkout parameters, 156
LC/MSD, 140
analysis study parameters, 96
checkout methods parameters, 144
data analysis, 135
importing expected masses from CSV, 106
preparing for checkout, 142
purity calculation parameter settings, 148
purity calculations, 97
reviewing checkout results, 152
setting data analysis, 92
setting sampler parameters, 147
signal selector/indicator, 126
software, 13
standalone software, 13
Liquid Chromatograph, Series 1100, 12

M

Maintenance mode, 145
flushing syringes and tubing, 34
initializing sampler, 32
replacing syringes, 36
Mass spectra, 101
checkout sample data, 152
data, 99
viewing results, 126
Masses
adding new lines to table, 103
background to subtract, 98
calculating from molecular formula, 104
importing using Excel spreadsheet, 106
Method & Run Control screen, 15, 16, 17, 114
Method, running next, 96
Method-related information, 40
Micro Plate Sampler, 12, 20, 140
preparing for checkout, 145
preparing for mass-based fraction collection checkout
, 177
preparing for UV-triggered fraction collection checkout, 157
software, 13
Microtiter plates, customized, 27
MSD Signals dialog, 94
Multiple plots, 48

N

Name format for samples, 45

Next method, selecting conditions for running, 96
Noise threshold, for purity calculations, 98

O

Open Study dialog, 55
Operator's name, entering, 45
Optimizing sampler performance, 88

P

Parameters
  dead volume, 22
  of study, 38
  syringe volume, 22
Paths
  directory, 23
  file, 23
  global, 23
  naming caution, 23
  previously acquired study, 23
  specifying manually, 23
Peak detection, 80
  parameters, 81
  parameters disabled, 110
  signal source for, 22
Plates
  extended, information on, 101
  extended, reports on, 100
  name in study, 45
  table for studies, 39
Plots
  horizontal, 48
  multiple, 48
  vertical, 48
Pooling, 115
Purity calculations, 97
  chromatographic separation, 99
  enabling, 98
  expected adduct ions, 97
  expected dimers, 97
  extracted ion chromatogram, 99
  FIA, 99
  for ADC data, 99
  for mass spectrum data, 99
  for UV data, 99
  LC/MSD parameter settings, 148
  noise threshold, 98
  reference, 99
  setting up, 97
  subtracting background masses, 98
  total ion chromatogram, 99
  user-selected adducts, 97
  user-selected isotopes, 97

R

Rack Configuration screen, 22, 24
Racks
  changing configuration, 24, 25
  composition, 22
  configuration caution, 22
  configuring for study, 39
  configuring system, 22
  creating new configuration, 24
  opening existing configuration, 25
  plate lists, 27
  removing, 25
  Select window, 25
  showing name in study, 45
  wrong type, 25
Report dialog, 133
Reports
  ChemStation Data Analysis, 100
  enable data analysis, 46
  Extended Plate, 48, 100, 133
  graphical, 101
  parameters to generate, 55
  print chromatograms, 48
  Summary Plate, 100, 133
Reprocessing studies, 113, 132
Results
  data from another method, viewing, 127

data in more detail, viewing, 125
data in other wells, viewing, 125
exporting, 133
graphical data, 125
printing, 133
review CC-Mode remotely, 135
review for individual samples, 125
review for study, 124
review remotely, 135
sample/fraction relationship, viewing, 129
Run Time Checklist dialog, 94, 113
Running
checkout analysis, 150
run next method if option, 96
UV-triggered fraction collection analysis, 162
Runs
monitoring progress, 117
monitoring status, 117

S

Sample Information dialog, 94, 112, 150
Sampler
centered loop fill task, 72
Collection mode, 16
constants, 22
draw from destination task, 78
enable fraction collection, 83
initialization, 32
inject sample task, 71
Maintenance mode, 32, 145
optimizing performance, 88
parameter settings for LC/MSD checkout, 148
parameter settings for mass-based fraction collection checkout, 171, 181
parameters for UV-triggered fraction collection checkout, 160
parameters task list, 109
preparing for mass-based fraction collection, 168
rinse injection port task, 75
rinse needle task, 75
setting parameters, 147
standard injection program, 84
status and configuration, 34
typical tasks, 84
wash vial task, 75
Sampler Maintenance screen, 32, 34
Sampler Only mode, 16, 20, 22
Sampler/Fractionator mode, 16, 20, 22
Samples
adding to study, 50
analysis, methods of, 43
importing data, 51
injection methods, 43
list for study, 39
name format, 45
order in CSV file, 52
order of selection, 50
parameters to analyze, 55
plate name in study, 45
rack name in study, 45
removing from study, 53
selecting for study, 50
way to name, 45
Screens
Method & Run Control, 16, 17, 114
Sample Information, 150
Sampler Maintenance, 32, 34
Status, 117, 150
Study Layout, 17, 145, 148
Study List, 149
System Configuration, 34, 36
Select Rack window, 25
Selecting expected adducts and dimers, 97
Shutting down, 45
Signal, 126
change, 22
delay, ADC, 22
source, 22
Signal selection dialog, 127

Signal selector/indicator, 126
Software
  CC-Mode, installing, 13
  CC-Mode, starting, 15, 17
  CC-Mode, uninstalling, 14
  ChemStation, 12, 13
  data analysis, 13
  fraction collection, mass-based, 13
  LC/MSD data analysis, 13
  Micro Plate Sampler, 13
  starting, 15
  uninstalling, 14
Specify Report dialog, 46, 94, 100, 113
Specify report format, instructions, 100
Start fraction, 80
Start study dialog, 115, 119
Startup sequence, CC-Mode software, 15
Status screen, 150
  information displayed, 117
  opening, 117
  status bar, 117
Stop fraction, 80
Studies
  adding expected masses to list, 102
  checkout layout, 148
  creating new for checkout, 146
  creating new from existing, 42
  creating new study, 169, 178
  creating, procedures for, 42
  data path field, 38
  database content, 39
  fraction list, 39
  information, 39
  layout for purity calculations, 148
  monitoring run status, 117
  opening, 115
  parameter content, 38
  parameters, 55
  parameters, modifying, 42
  pausing, 121
  plate table, 39
  pooling option, 115
  rack configuration, 39
  reprocessing, 113, 132
  reprocessing caution, 132
  review results, 124
  review results, individual samples, 125
  running, 115
  sample list, 39
  save as, 56
  setting data analysis parameters, 95
  setting parameters, 147
  setting up new, 41
  starting, 115
  stopping, 121
  system checkout lists, 149
Study
  defined, 38
  previously acquired, 23
Study Layout screen, 17, 44, 145, 148
Study List screen, 149
Study List sorted by sample Location dialog, 128
Study parameter dialog
  analyzing fractions, 119
Study parameters dialog, 43, 44, 55, 57, 95, 97, 109, 135, 147
Summary Plate report, 100, 133
Syringes
  flushing, 34
  new size, 36
  replacing, 36
  setting volume, 36
  volume, 34
System checkout procedures, 140
  column required, 141
  instruments required, 140
  sample required, 141
  software required, 141
  solvents required, 141
  study lists, 149

System configuration, 20
    changing, 21
    in CC-Mode remotely, 135
System Configuration screen, 34, 36

T

Tabular View dialog, 125, 130
Time delay, ADC signal delay, 22
Time-based collection, 80
Total ion chromatogram
    checkout sample data, 152
Total ion chromatogram (TIC), 99

U

User selected adducts and isotopes, 97
UV chromatogram
    checkout sample data, 152
    purity calculation data, 99
    viewing results, 126
UV-triggered fraction collection, see Fraction collection, UV-triggered

V

Vertical plots, 48
View Graphical Sample Data dialog, 125, 127, 130, 152

W

Warning during CC-Mode software installation, 14

Z

Zone ID, 44
Zone subdirectory, 45, 46

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